DRUM *and* CROAKER

A Highly Irregular Journal for the Public Aquarist

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Cover Photo: *Lyrocteis*, Bart Shepherd, California Academy of Sciences
Interior Gyotaku: Bruce Koike (pp. 17, 25, 33 & 75).
Interior Line Art: Craig Phillips, D&C 1969 (2), etc.

DRUM AND CROAKER ~50 YEARS AGO

Richard M. Segedi

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Excerpts from the 1969 issues, Edited by Craig Phillips, Assistant Curator, National Fisheries Center and Aquarium, Washington, D.C.

REMOVAL OF AMMONIA FROM AQUARIUM WATER BY CHLORINATION AND ACTIVATED CARBON

By John Charles Baummer, Jr. and Loren D. Jensen

It was found that in the process of chlorination followed by activated carbon treatment, about 10 parts by weight of chlorine is required to oxidize 1 part of ammonia-nitrogen when the reaction time allowed is 15 minutes. The products of the reaction are gases which are eliminated from the water. Thus, eutrophication is avoided.

OBSERVATIONS MADE DURING THE BREEDING OF CUTTLE FISH

Werner Schroeder Director, Berlin Aquarium

At the end of April 1965, the Berlin aquarium received an 18 cm *Sepia* which had been caught two days earlier on the northern coast of Sicily. The animal, which had arrived without injuries, was put in display tank holding 4,500 liters and was kept in artificially prepared sea water of a density of 1,025 and at a temperature of 200C. The second day we found more than 100 dark—grey eggs on a celluloid aeration pipe. The first young animals hatched seven days later. The time of hatching varied greatly, the last animals only hatched after 3 months.

BOOKLET ON LARGE AQUARIA

Earl S. Herald, Steinhart Aquarium

A new manual entitled *Some Guides to Designing, Building and Operating Salt Water Aquarium Systems* is available free of charge to workers seeking assistance with marine systems ranging from 100 to 100,000 liters in capacity. This booklet gives information on methods of construction, control of physical, chemical, and micro-biological factors, and ecological and health conditions in large aquarium systems.

THE TRAINING OF FISHES

Shiro Takamatsu (Edited by James Atz)

About seventy species, more than one thousand fish, were kept in twelve aquariums, which ranged from 1.4 to 30.3 (metric) tons in water capacity. Thirty seconds before feeding, each aquarium was lit by a 500-W flood-light, 80 cm above the water surface. At the time of feeding, which took place every other day, this conditioning was repeated over and over for about four months. As a result, almost all the species in the aquariums had been conditioned completely by the end of the fourth month.

Now the fish gathering toward the flood-light are caught in a polyethylene basket without showing any signs of injury or fright; if there is some food in the basket, they are willing to get into it. No matter how many times we transfer a fish, it always gathers under the floodlight whenever it is turned on. Needless to say, even under the flood-light, we have to be very careful not to harm any fish at the time of transferal. In this way, we have been able to transfer about 70% of the entire fish population into a tank of 30 metric tons water capacity at the same time, without damaging any of the fishes.

CRAIG PHILLIPS, Assistant Curator, NFCA, has assumed the direction of the National Aquarium *(Washington, D.C.).*

LOUIS GARIBALDI, formerly with the Steinhart Aquarium, San Francisco, is now Curator of Fishes at the new American Broadcasting Company's Marine World at Redwood City, south of San Francisco on the bay.

TAX ENIGMA; EXCHANGE and SALE versus GIFT
Earl S. Herald, Steinhart Aquarium

In this State if you exchange fishes or other animals with another non-profit institution, dealer, or friend, you must pay a sales tax on the market value of the animal. But a gift is non-taxable. At Steinhart Aquarium we do not sell salt water which is taxable, but we do charge a pumping service fee which by odd coincidence is equivalent in amount to that charged earlier for salt water before we were enlightened.

What is needed now is a new term in the English language which will imply an open gift of a living animal without any visible strings attached. Please note that a thought process is non-taxable. Thus, the recipient, after admiring his new acquisition, then suddenly feels compelled through mental telepathy communication to present you with something of at least equivalent value. The tax-free term for this sequence, that might in an unguarded moment have previously been called "exchange," is now recognized under a newly-coined word, TRANSBAR. May all of your tax problems henceforth be transbar.

2019 EDITOR'S NOTE: 50 YEARS OF DRUM AND CROAKER – SORT OF

If this is Volume 50, why don't the "50 Years Ago" pieces now harken back to the original 1958 issue? Alert readers will note that while this is the 50th year where an issue was produced, Drum and Croaker has actually existed for 61 years. What happened? There were a number of 1-2-year gaps in publication, and one large gap of ~5 years from 1958 to 1993. This year's "50th Anniversary" milestone is therefore something of an artifact.

Happy 50! or 61! - Pete Mohan, Editor

COLLECTING AND EXHIBITING *Lyrocteis imperatoris* Komai 1941, A SESSILE CTENOPHORE FROM MESOPHOTIC ECOSYSTEMS

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Biology and Ecology of *Lyrocteis*

The phylum Ctenophora consists of gelatinous organisms that are found in nearly all marine ecosystems. The majority of ctenophores are free-swimming or planktonic. However, one order, Platyctenida, contains adult forms that live attached to other animals or the substratum (Brusca et al., 2016). These include a few sessile species that are occasionally reported as commensal organisms in aquariums, such as *Coeloplana* spp. (Sprung and Delbeek, 1997). The order Platyctenida also includes two species within the genus *Lyrocteis*.

Lyrocteis imperatoris Komai, 1941 was described from specimens dredged from mud bottoms at a depth of 70 m in Japan. The first record of *L. imperatoris* outside Japan was published in 2009, when two specimens were found in relatively shallow water (18-22 m) in Korea (Song and Hwang 2009). A second species, *Lyrocteis flavopallidus*, was described from individuals collected between 36 and 55 m depth at McMurdo Sound, Antarctica (Robilliard and Dayton, 1972). Single individuals of *L. imperatoris* have been spotted on occasion on mesophotic ecosystems in Palau and Pohnpei (R. Pyle and B. Greene, pers. com.). In contrast, we found high concentrations (up to 12 individuals per square meter) of *L. imperatoris* on a mesophotic coral ecosystem in Bauan, Philippines, during expeditions in 2014, 2015, and 2016 (Shepherd et al., 2018a). These were clustered together in groups, often elevated above the substratum, "perching" on abandoned fishing line, gorgonians and black corals (Figure 1). Over the course of three sequential years, we collected 24 *L. imperatoris* from depths of 75-110 m for research and educational display at the Steinhart Aquarium.

Lyrocteis has a lyre-shaped body composed of a saddle-like trunk with two arm-like processes on either side. The external margin of the body is encircled by a deep furrow. Feeding tentacles are extended from the distal end of the marginal furrow on each arm, and food is moved along the furrow to the mouth, which sits at the underside of the trunk (Komai, 1941; Soon and Hwang, 2009). The oral area of the trunk is used to hold on to the substratum, and can be expanded into a "skirt" or "sole", which can also be used for moving (Robilliard and Dayton, 1972; Soon and Hwang, 2009; Brusca et al., 2016). *L. imperatoris* occurs with a wide range of colors: milky-white, mustard yellow, bright pinks, purples and reds, and blue-black. Often, the marginal furrow and bands along the genital tracts are a strikingly different color than the ground color of the body, and some specimens are spotted.

Figure 1. An aggregation of "angry bunnies": the sessile ctenophore, *Lyrocteis imperatoris*, as seen in its natural habitat (Bauan, Philippines), elevated on a gorgonian at approximately 90 m depth. Photo: Bart Shepherd.

Lyrocteis is a simultaneous hermaphroditic brooder and presumably can self-fertilize (Brusca et al., 2016). Embryos are held in specialized internal brood chambers, and individuals may contain several hundred embryos at various stages of development (Komai, 1942; Yamauchi et al., 2017). The embryos emerge by piercing the parent's epidermis during hatching (Komai, 1942). Platyctenid ctenophores are reported to be able to reproduce asexually through fission, although this has not been specifically observed in *Lyrocteis* (Brusca et al., 2016).

Public aquarium exhibits of ctenophores are usually restricted to the planktonic species. Prior to our work with *L. imperatoris*, aquarium exhibitions of sessile ctenophores occurred only in Japan. Both Aquamarine Fukushima and Okinawa Aquarium have displayed *L. imperatoris*. In late 2014, Aquamarine Fukushima collected two individuals of *L. imperatoris* from depths of 133-142 m using a ROV. The specimens were brought back to the aquarium, where they released nearly 200 larvae, many of which were subsequently raised to produce additional adult specimens (Yamauchi et al., 2017). Okinawa Aquarium has also collected adult *L. imperatoris* and raised the larvae (Yamauchi, pers. com.).

Our efforts with specimens from the Philippines were conducted, unbeknownst to us, at roughly the same time as those of our Japanese colleagues. Collecting and shipping methods, care and husbandry of adult individuals, aquarium behavior, and methods for eliciting release of brooded larvae are described in this paper. We had two main goals for this work: first, to study the ecology and life history of *L. imperatoris*, and second, to feature the species as a charismatic ambassador for mesophotic coral ecosystems in our exhibit, *Twilight Zone: Deep Reefs Revealed*, which opened in June 2016.

Collecting *Lyrocteis imperatoris* in the Philippines

In 2014, we began conducting dive surveys of mesophotic coral ecosystems (30-150 m) in the Verde Island Passage, Philippines, a geographic region considered to be the "center of the center of marine biodiversity" (Carpenter and Springer, 2005). Divers use closed-circuit, mixed-gas rebreathers to reach working depths of up to 150 m. Our work is typically conducted by diving in teams of three to five individuals, with each diver assigned a specific task: capturing fishes, collecting benthic invertebrates, taking photographs or video, or surveying fishes along transect lines. During the 2014 expedition, we opportunistically collected two specimens of *L. imperatoris* at approximately 75 m depth, placing them in an early version of the Submersible Chamber for Ascending Specimens (SubCAS), our portable, submersible fish decompression chamber (see Shepherd et al. 2018b for details on this device). Not knowing what these brightly-colored gelatinous blobs were, we brought them to the invertebrate zoology team from the California Academy of Sciences for identification.

The following year (April 2015), we collected three individuals at approximately 85 m depth in order to study their care and life history in aquaria, and to explore their potential for a new exhibit we were planning. These specimens were collected in plastic deli-type food containers (approximately one liter). Prior to shipping, they were held for a few days in a 220 L polyethylene barrel of gently aerated natural seawater at 18-20°C. The ctenophores were packed in two-liter plastic bags completely filled with seawater, placed in styrofoam coolers with cardboard outer liners, and shipped via air cargo from Manila to San Francisco (approximately 24 hours transport time). Fist-sized pieces of ice wrapped in newspaper were used to keep the water in the shipping containers chilled during transport. HOBO® data loggers in the boxes tracked the temperature, showing a slow, steady decline from 21°C to 16°C between packing and unpacking.

In May 2016 we conducted a collecting expedition to the Verde Island Passage for various targeted species of fishes and invertebrates in preparation for the opening of our exhibit, *Twilight Zone: Deep Reefs Revealed*. This included *L. imperatoris*, which was to be a signature species for the exhibit, showcased in a dedicated aquarium at the entryway to the gallery. On this trip we collected 19 individuals from depths of 75 to 90 meters. All of these specimens were held in the field and then shipped to Steinhart Aquarium using the methods described above, with 100% survival.

We returned to the Verde Island Passage in May 2017, with the specific goal of obtaining more individuals of *L. imperatoris*, both for our displays and to attempt to close the life cycle. Curiously, populations that had been robust for the prior three years were nearly completely absent. We found only three individuals, and they were all shrunken or otherwise deformed. This remarkable change in population density led us to describe this unique concentration of ctenophores as an "ephemeral aggregation" (Shepherd et al., 2018a). Three suboptimal specimens were collected and shipped to Steinhart Aquarium, where attempts were made to elicit the release of brooded larvae with the hope that we might be able to grow these into adult individuals for future display.

Collecting such delicate gelatinous animals while diving proved to be fairly difficult. Our early attempts to place them into hard plastic containers (e.g. the SubCAS chamber, plastic deli cups) were challenging. The animals are so lightweight and fragile that they were nearly

impossible to manipulate underwater, and they were easily damaged by the edges of the containers. This was complicated by the fact that we were working at extreme depth with very limited bottom time. On our second expedition, in April 2016, we discovered an easy and reliable way to collect and handle *Lyrocteis.* Employing the strategy that responsible dog owners use to clean up their pets' feces, we inverted a clear plastic bag over one hand, wrapped it around the ctenophore and its perch, clipped the perch free from the substratum, and then secured the bag with a rubber band. Many specimens were found clinging to abandoned monofilament fishing lines; these were collected by clipping the monofilament free and putting both it and the attached animals into a plastic bag.

Aquarium Care of *Lyrocteis imperatoris*

The three individuals from the 2015 Philippines expedition were managed in behind-the-scenes support areas at Steinhart Aquarium. They were held under subdued, ambient lighting in a 100 L pseudokreisel tank with silicone air tubing provided as perches, maintained at 20°C, and fed with *Artemia* nauplii, frozen mysids, and krill. At five to six months, two of the specimens began to shrink, become less active, and no longer feed. The third specimen lived about 11 months before declining in the same manner.

The 16 specimens collected in April 2016 were managed on exhibit in the newly opened *Twilight Zone* gallery (Figure 2). They were held in a 750 L rectangular acrylic tank as part of a larger, 1,900 L system composed of six tanks. The system was maintained at 20°C. The *Lyrocteis* aquarium was outfitted with a curved plastic (KYDEX®) backdrop and two water inlets in the front corners of the tank, which created a flow pattern similar to a horizontal stretch kreisel. Lighting was provided by an Aqua Illuminations Prime LED unit, weighted toward blue wavelengths to simulate lighting at depth. The majority of the top of the exhibit was shaded with an opaque plastic lid that restricted direct lighting to a small area over the center of the tank. Decor included a coral sand bottom and several pieces of aragonite reef rock. The tank also contained living black corals, which were used as perches.

Adult *Lyrocteis* were fed a variety of frozen foods. Larger food items (pieces of fresh fish or shrimp) were also readily accepted. During feeding, tentacles are rapidly retracted, carrying the food to the marginal furrow, where it is then transported to the mouth. Amazingly, they are able to untangle their feeding tentacles from those of other individuals, despite appearing to be one giant sticky web.

Lifespan in aquaria ranged from five to nine months, with a few individuals living as long as 11 months. Yamauchi et al. (2017) maintained wild-collected adults at Aquamarine Fukushima for between 31 and 87 days. During this time, one of their specimens released nearly 200 larvae over a period of 49 days. Many of these larvae were raised to produce additional specimens. The longest they have kept a single individual alive is about 180 days (Yamauchi, pers. com.). Because their wild-collected adults ceased to feed and began to degrade after releasing larvae, Yamauchi et al. (2017) inferred that *L. imperatoris* breed during a single phase of their life, and that they die following release of larvae.

The *Lyrocteis* display at Steinhart Aquarium proved to be very popular with the public. A social media campaign, where we jokingly called them "sea peeps" and "angry bunnies" created a

lot of engagement. Despite initial doubts from leadership, marketing and exhibit designers, the animals proved to be immensely popular and engaging, proving that invertebrates can in fact compete for attention with charismatic megafauna.

Behavior and Reproduction

Lyrocteis exhibited several interesting behaviors in our aquaria. As noted in the literature and observed in our field expeditions, they often elevate themselves above the substratum. The first known record of this animal, written in 1896 when it was pulled up on a longline, includes a drawing showing it firmly attached to a gorgonian (Komai, 1942). Robilliard and Dayton (1972) found *L. flavopallidus* on sponges, and noted that the ctenophores somehow found and crawled up steel rods that were placed in the substratum to mark their positions. Song and Hwang (2009) collected their specimens from ropes. Other studies found *L. imperatoris* on sunken sperm whale carcasses in Japan (Fujiwara et al., 2007). Yamauchi et al. (2017) collected their specimens from gorgonians. In the Philippines, we routinely saw them attached to abandoned fishing line, black corals, and gorgonians (Shepherd et al., 2018a). In aquaria, they perched on gorgonian skeletons and live black corals, and occasionally could be found on rocks or on the sides of the aquarium (Figure 2).

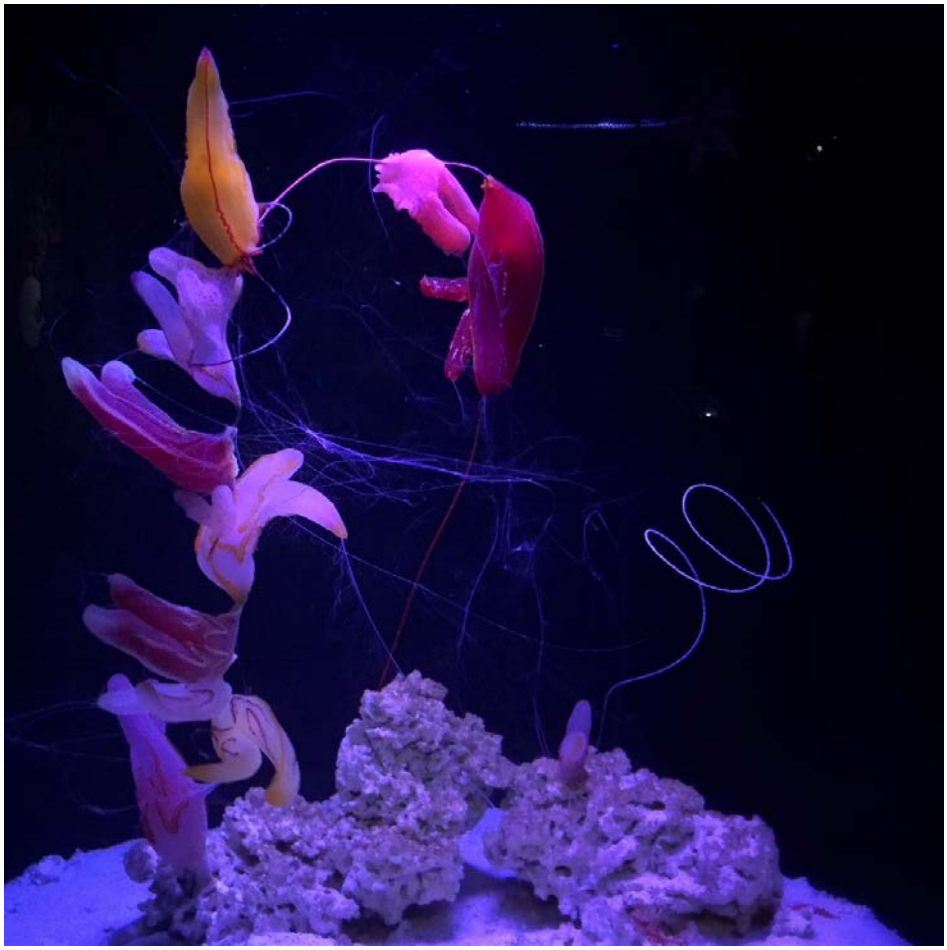

Figure 2. The *Lyrocteis imperatoris* aquarium in the *Twilight Zone: Deep Reefs Revealed* exhibit at Steinhart Aquarium, California Academy of Sciences. Photo: Bart Shepherd.

Although *Lyrocteis* is able to crawl quite effectively using the sole or skirt at the base of the trunk, we also witnessed them using their feeding tentacles to reach out and attach to neighboring structures and reel themselves in, kind of like Spider Man! This aquarium observation supports what Robilliard and Dayton (1972) observed in Antarctica - *Lyrocteis* appears to use its tentacles to explore the surrounding environment and locate structures where it can elevate itself above the substratum. *Lyrocteis* exhibited negative phototaxis; the animals moved away from the overhead aquarium lighting. This was especially the case when we shifted the color spectrum of the LED fixture toward the red/orange wavelengths in an attempt to highlight the ctenophores' dramatic colors. Within 24 hours, all individuals had migrated toward the bottom of the aquarium away from the light.

On 22 May 2015, roughly one month after collecting our first group, we observed a small section of tissue from one of the *Lyrocteis* that appeared to have been produced by fission or budding (Figure 3). This small specimen in some ways resembled adult *Lyrocteis*, including having a skirt or sole used for attaching itself, and (perhaps) the beginnings of the arm-like appendages. After a few days, this small specimen had vanished. We present this as evidence that *Lyrocteis*, like other platyctenid ctenophores, is capable of asexual reproduction by fission. Asexual reproduction may also explain the high concentration of similarly-colored individuals we found in the Philippines during the 2014, 2015, and 2016 expeditions.

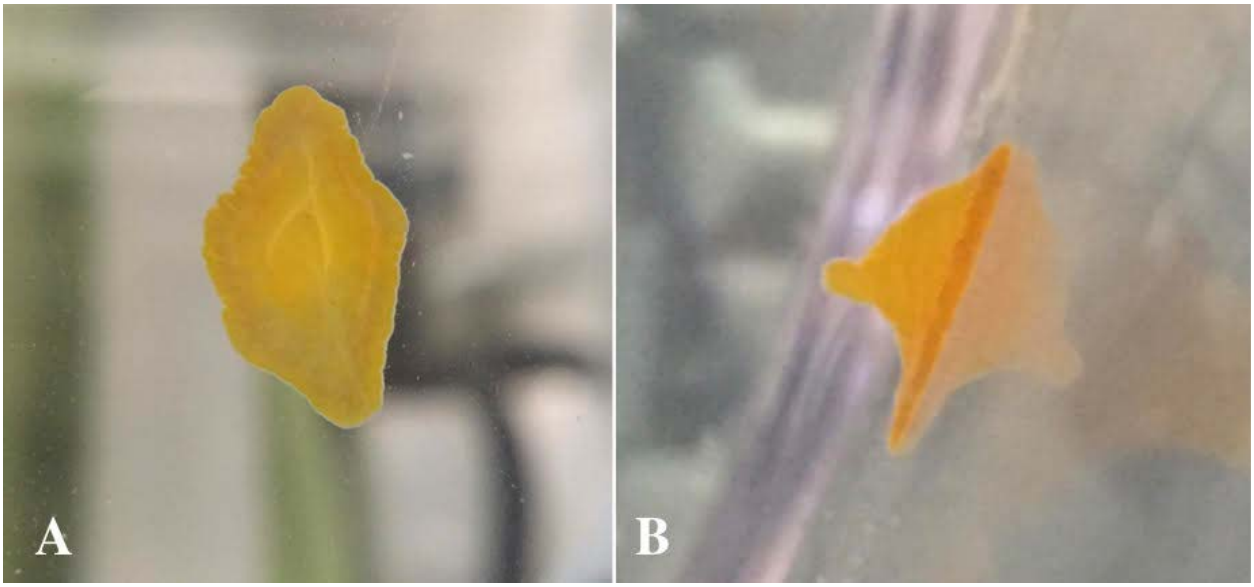

Figure 3. Other genera of platyctenid ctenophores are able to reproduce asexually via fission. Our experience suggests that *Lyrocteis* has this ability. We observed a small section of tissue separate from one of the adults in our holding system and behave as a unique individual. (A) view of base of the animal through the aquarium window, (B) lateral view of the same individual. Photos: Bart Shepherd.

Our final group, collected 17 May 2017, was placed in a 100 L pseudokreisel tank. Because these individuals were all damaged or deformed, and not really exhibit-quality specimens, we decided to keep them behind the scenes and attempt to elicit the release of larvae through photostimulation. They were held in complete darkness for a period of six hours, followed by exposure to direct incandescent lighting for two hours. This resulted in one *Lyrocteis* releasing at

least 47 larvae over a period of six days, after which it died. The larvae were transferred by pipette to a separate 9 L pseudokreisel tank on the same water system, and reared over the course of approximately 15 days. The population decreased by three to five individuals daily, but the cause of these losses is unknown as remains were never observed. The larvae that our individual released were rounder and almost half the size than those documented by Yamauchi et al. (2017), with body width and height approximately 1 mm versus approximately 2 mm. Both our larvae and those at Aquamarine Fukushima had four pairs of comb plate rows which were used for active swimming, a single sensory organ, chromatophores, and two sets of branched tentacles (Figure 4).

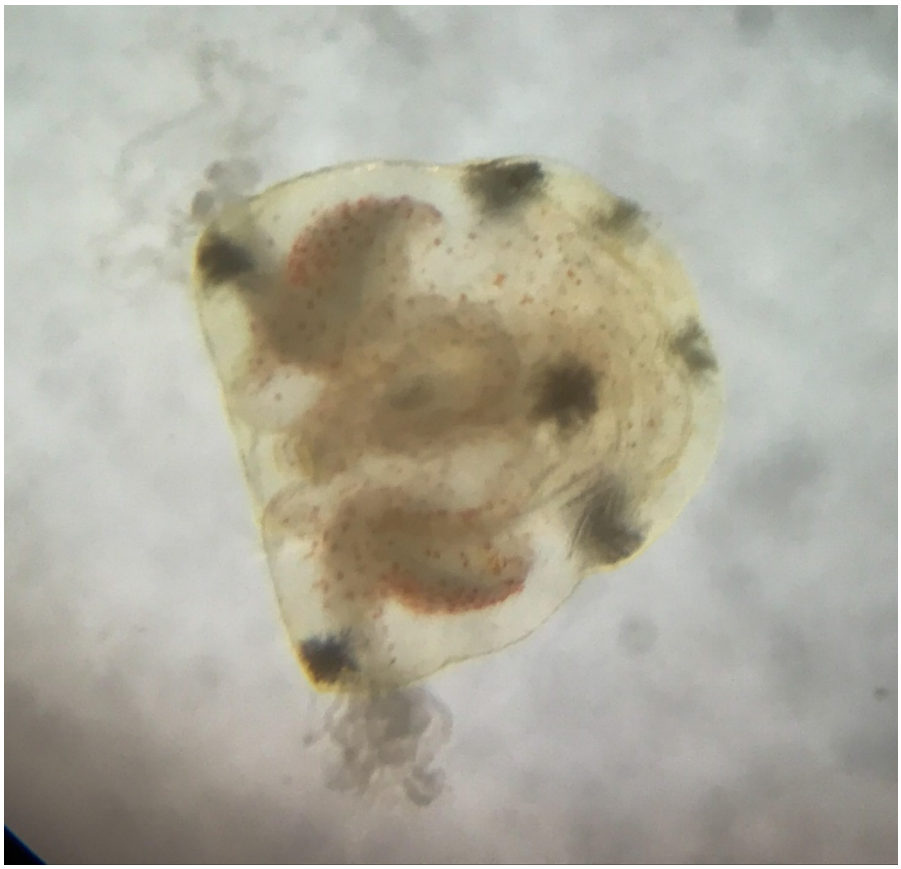

Figure 4. Larvae of *Lyrocteis imperatoris* released following photostimulation. Body width is approximately 1 mm. Photo: Steven Yong.

Due to the recent advances in jelly and pelagic ctenophore culture, plus the successful rearing of Yamauchi et al. (2017) of their larvae, we believe that there is potential for a culture program for *Lyrocteis*. A successful culture program would also ensure a stable aquarium population of these animals, which is important considering the resources required to collect them, and what may be seasonal or cyclical population levels in the wild. Should we have future opportunities to work with the species, we would like to duplicate the release of larvae through photostimulation to confirm its efficacy, as well as try different methods in rearing.

Acknowledgements

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A NOVEL METHOD OF ADMINISTERING DRUGS TO FREE SWIMMING LARGE AQUARIUM FISH AT USHAKA SEA WORLD

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Abstract

Administering drugs to fish in the aquarium by pressurized dart requires less effort from staff compared to other methods, and further it has shown to be positive for the target fish and the overall exhibit occupants. Capture damage to the individual fish and overall stress to all the fish that are in the exhibit is minimised. A new darting method is described which uses a modified spear gun to deliver the pressurized dart with the prescribed medication.

Introduction

Moving fish from a large multi-fish enclosure for husbandry reasons can physically damage individuals and can have a disruptive effect on other fish in the enclosure. In the past, in order to remove fish from any of our large multi-species exhibits, divers would enter the exhibit with barrier nets and hand nets to attempt to catch the target fish. This often resulted in physical damage and stress to all occupants of the exhibit. The darting method described requires less staff and significantly reduces the stress level of all the animals in the exhibit. This method has been successfully used on 17 different species of fish in four of uShaka Sea World's seven large exhibits.

The prescribed tranquiliser drug dose is determined by our Vet and is placed into a pressurised dart that is loaded into a modified speargun apparatus to be administered to the individual fish. Once the drug has taken affect the fish capture requires little effort, and in turn is presumed less stressful because there is little or no chasing of the fish.

Overall results have been positive for the fish, in that there is minimal physical damage and handling is reduced. Fish undergoing the darting and relevant medical procedure have been observed feeding again 48hrs later.

Materials and Methods

A Rob Allen 700mm rail gun was used for this purpose because accuracy with a short spear gun is better than a longer spear gun of 1000mm or more. An 8mm (outside diameter) hollow carbon fibre rod (1020mm) was modified to be the spear. It is lighter than a steel spear and would thus minimise the impact on the fish when shot. The back of a typical steel spear (the trigger locking section) (Figure 1) was modified to fit inside the carbon fibre rod (spear) and epoxy glued together. It is important that these two pieces are perfectly aligned to make a spear total length of 1120mm.

Figure 1. Trigger locking section of a typical spear.

A 120mm Length of high-density polyethylene (HDPE) was machined on a lathe to 20mm diameter for the front of the modified spear which holds the Paxarms remote syringe dart (Figure 2a). We use Paxarms darts but any system can be used. The housing is drilled out to suit the spear on one end (8mm inside diameter and drilled to a depth of 28mm to accept the carbon fibre spear) and the opposite end is drilled out to suit the syringe dart (13mm inside diameter and to a depth of 78mm to accept the dart) to create a sliding fit. Four holes are drilled (82mm from syringe dart insert) through the syringe dart seat allowing the dart in the housing to break suction when administering (Figure 2b). The two ends of the HDPE housing are machined to a taper for hydro dynamic relief.

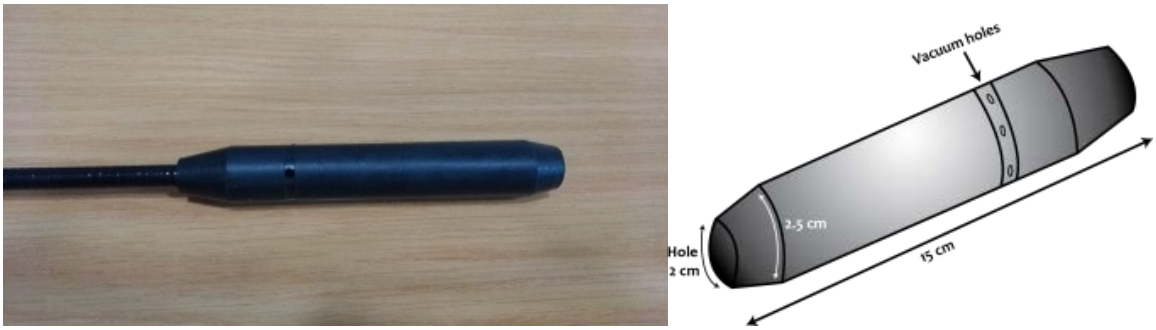

Figure 2. a) Machined HDPE chamber mounted on carbon spear shaft. B) Chamber details and dimensions.

A speargun 'rubber' (9mm outside diameter and 5 mm inside diameter) is used to propel the spear. With our setup the rubber is 600mm long (un-stretched) and is joined together with a "wish-bone" to create a loop (Figure 3). A 7kg pull was measured as the strength of the rubber when loaded, this is weaker than a typical spear rubber to further minimise the impact on the fish when shot.

The Paxarms darts (Figure 4) are known as syringe darts, and are made from a special polycarbonate material. The plunger in the syringe separates the drug chamber from the air pressure chamber. The darts also have a special needle with a side port.

Figure 3. Speargun rubber (top) with custom spear and Rob Allen 700mm rail gun.

The drug is injected into the drug chamber, and the needle with the sealing sleeve placed over the side port is attached. The sleeve stops the drug from squirting out once the chamber is pressurised. A normal syringe, filled with air, is used to pressurise the air chamber, and a one-way valve stops the air from escaping the chamber. The dart is now charged, and placed into the spear gun fitting.

When the dart hits the fish, the needle penetrates the muscles, and the skin will push the sealing sleeve backwards over the needle. This will open up the side port. Now that the port is open, pressure can be released, and the plunger, with pressurised air behind it, will move forwards, injecting the drug intramuscularly. The dart either drops out by itself, or will need to be removed once the fish is sedated and caught.

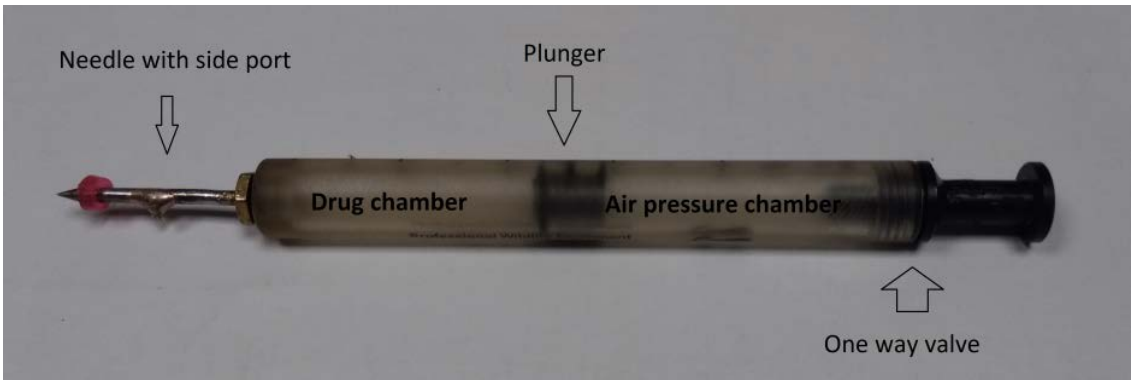

Figure 4. Paxarms projector dart details.

The pressurized syringe dart is sized according to the volume of drug/s to be administered. The syringe dart needle is selected (length/volume, diameter and number of barbs) according to the fish that will be targeted. Soft skin fish species need needles with two barbs to keep the dart in

the fish long enough to allow the drug to be absorbed into the fish. Large scaled fish species only require a needle with one barb (Figure 5). The length of the needle depends on the lateral width of the fish. The diameter of the needle depends on the size of the fish, larger fish need larger needles.

Figure 5. Single and double-barbed needles.

Once the pressurized syringe dart is prepared, it is placed into the HDPE holder with some lubricant, to allow it to easily slide out of the housing after it has penetrated the fish. From our experience it is preferable that the dart stays in the fish to ensure that 100% of the drug is administered and no assumptions are made as to how much drug has leaked out.

Successful darting of the target fish requires that the diver has a good understanding of its behaviour (including how fast the fish swims, does it have a specific swim pattern, is it skittish, etc.). Snorkelling during this procedure is advised as there is less underwater noise, compared to when using SCUBA on the approach to the fish. The fish is approached to approximately 1 m with arm extended straight, the area below the dorsal fin is aimed at (the size of this area depends on the size of the fish) (Figure 6). Once darted the fish is observed until obvious signs of the drug effects are noticed and the fish is swimming slowly. Depending on the drug(s) used and the fish sensitivities, such as weight, species, and health condition, a second dart may need to be administered if no signs of affects are noted after approximately 30 min. The fish can easily be caught with a soft plastic net or a plastic sock. Once the necessary move or medical procedure is completed, a reversal drug can be administered and the fish is released again. The fish is then monitored for its recovery behaviour. This is also important to assess the species response/sensitivity to the drug administered and if the dosage used was correct. Good record keeping for these procedures are important. Information that is recorded includes: species, delivery of dart success; drugs used; dose used; behavioural response of fish; duration of procedure; etc.

Figure 6. Darting technique.

Drugs:

Ketamine 4mg/kg; Medetomidine 0.2mg/kg; Butorphanol 0.01mg/kg
Reversal for Medetomidine – Atipamezole 1mg/kg

This concentration and mix of drugs has been our most broadly used mix across the 17 species with the most successful sedation response. We have in certain cases used slightly stronger doses once we have a history of a species with regard to this sedation method. We Suggest conservative approach to build experience.

Results

This drug administration method has allowed us to catch, handle and treat individual fish from a multi species exhibit with less stress and physical damage to both the targeted fish and the other inhabitants of the exhibit. A total of 161 of 17 species darted between November 2015 and October 2018 (36 months). The weights of the individual fishes ranged from a 2kg Zebra fish (*Diplodus cervinus hottentotus*) to a 20kg Southern Pompano (*Trachinotus africanus*). All procedures where fish were darted with this method at uShaka Seaworld have been successful and no mortalities have been experienced.

Discussion

Even though a similar technique has been reported on before (Harvey et al., 1988), this method is different and unique. It has greatly changed the way our team removes fish from large exhibits.

It has been very interesting noting the variance in drug tolerance across the different species of tropical fish, as well as the target fish behaviour during the darting procedure i.e. the target fish will be the only fish keeping its distance, whilst other fish swim calmly past you. We have found that reef fish species require a higher dose of sedative than the pelagic species. As the pelagic species seem to be more sensitive to the drugs. We have found that individuals react differently within species as well.

I believe this method has huge potential in public aquariums as it reduces stress, time, effort, and physical damage of free-swimming large aquarium fish during moves out of multi species exhibits.

Acknowledgements

The author would like to thank the SAAMBR team for all the support given in the process of design and success of this process. A special thanks to Matt Myhill; Dr Francois Lampen; Sr. Marlé Benade; Cornelius Koekemoer of uShaka Sea World for their in-depth input and time.

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ON GIVING THE APPEARANCE OF SOLIDITY TO PURE WIND

An Aggravated and Anonymous Aquarist

(Editor's Note: For some historical perspective, also see: Sieswerda, Paul. 2005. Of Sea Stars and Jellyfish. Drum and Croaker 36: p3.)*

As a community the animal care profession has achieved an unparalleled sea change as of late that has gone largely unnoticed. For the first time since the mid 1800's when the first public aquaria were established, we have not a single fish in captivity! Did all the fishes suddenly make a Nemo-esque escape to the sea? Are they in witness protection? Were they abducted by aliens?

I know there are those reading this in disbelief, but rest assured, animals at the fashionable facilities are no longer *captive*, they are *in human care*. What exactly is the difference, an inquiring reader might ask? Well to be brutally honest there is absolutely no difference whatsoever, but in the tradition of good old American bureaucracy why use one word when you can use three! But more importantly when did the terms captive or wild become *verboten*? To take an even deeper look in the mirror why did we as a collective group of professionals, allow such a ludicrous example of lexicological gymnastics to be foisted upon us?

When did animals stop being *captive* and start being *in human care*? Did this happen chronologically before or after the great mass starfish extinction of the late 20th century or the disappearance of the world's jellyfish in the early 21st century? When did the use of plain language take on an almost Orwellian tone in our industry, with terms like tank and captivity becoming *doubleplusungood*? The zeal with which some have sought to stamp out plain language amongst highly educated people in our industry in recent years has been unsettling to those who value straightforward and effective scientific communication.

This lexicological hydra rears its ugly head periodically never to be slain for good; while the supreme court may have never had to hear arguments in Pomacentridae v. United States (e.g. *D&C 2005 – "…save the Jellyfish before it's too late or we may find the Supreme Court deciding if Angelfish can be taught in public schools]"), the cause of modification of free and common expression is intermittently taken up by some in our field as the *cause célébre*. While the current standard-bearers undoubtedly think this is the best way forward, if one stands back and views the current campaign to mold our language in greater context this attempt to reshape 'captivity' into 'human care' and the like appears to have all the hallmarks of a fool's errand. Now, more than ever we need effective communicators in STEM education if we are to establish ourselves as moral authorities on conservation and stewardship.

Ultimately will those dedicated to cloaking simple language in euphemism and jargon find that they have advanced our cause, or will they look back at a circular path traveled that has brought them to the same point where we now stand? Novelist Kurt Vonnegut once wrote that "any scientist who couldn't explain to an eight-year old what he was doing was a charlatan", and this is spot on in the realm of science education and communication. The average visitor (especially children) has a limited attention span, and our interactions with them are ever so brief, that when an honest question is posed and the reply is a pedantic correction such as "actually, our animals live in habitats not tanks" or the like, we have lost a fleeting and precious opportunity to educate, inspire, or shape a budding opinion.

Above all to maintain the moral high ground we must avoid faults in logic as we make our case, and not stoop to the same level as our detractors. As one example we decried the disingenuous tactics employed by our adversaries when they tried to "re-brand" fishes as "sea-kittens", and we did so with conviction as not only was this a frivolous exercise, it was based on unsound reasoning. The appeal to emotion is a well demonstrated fallacy in classical logic in which an emotional response is manipulated in lieu of a valid argument. In this instance we recognize that our detractors are playing "dirty" yet, here we are, attempting to do the same thing by using euphemisms as a supposed panacea to make our argument more palatable…will the public genuinely forget their visceral reaction with a simple change in terminology? If we are to assume the public is so easily swayed then we are all putting far too much effort into our crusade to inspire the next generation towards conservation. If only it were that easy!

As animal professionals we are at our core scientists. Scientists have long struggled, albeit with good intention, to communicate the significance of our work to the masses because we often do not speak plainly and obscure the fundamental truths of our field with jargon. Yet today our industry is intentionally using language to obfuscate meaning in an attempt to distract the reader, thinking we can "reset" the conversation from a more equal starting place.

If critics of our profession claim injustice exists by keeping animals in tanks, and our argument places more emphasis on the fact that they are not "tanks" they are "habitats" than on the rationale for keeping those animals, then like it or not we have ceded the argument to our opponents by refusing to engage plainly. Likewise, if we insist, screeching and wailing, that animals are not kept in "captivity" but rather are "in human care" we have again ceded the argument to our adversaries. To attempt to change thought by changing language rather than by citing facts is fundamentally dishonest, and we do not give our audience (or our opponents) enough credit. We gain nothing by "reframing" the argument in language designed to distract and obfuscate meaning. Captive is the antonym of wild. I can defend to the public why species as a whole can benefit from their conspecifics being in captivity in an aquarium, but this position is undermined when trust is lost because our community has not spoken with one singular voice, and others have diluted our credibility with pedantic euphemisms and circumlocution. The public, both those who oppose us and those we have yet to convince, can sense when we are being disingenuous; and at this crossroads we haven't the time to debate semantics over substance.

The burden of proof to sustain our institutions through the 21st century and beyond lies with us. We must make the case in the public eye that aquaria and zoos are legitimate centers of scientific inquiry, education, and conservation and not Victorian menageries to be gawked at by the masses as a source of entertainment. The people that live in our communities (whether they visit our facilities or not) are at a crossroads where popular opinion is being shaped as to the role and the future of our profession. We must not presume to talk down to them or change the conversation as we make this case. The public at large is more intuitive than most of us give them credit for and they can discern when they are being manipulated. If we insist on trying to shape thought under the guise of "framing the conversation" or "branding" ourselves rather than changing perceptions through having an honest conversation we will incite distrust in our community as a whole, and the public will regard us with the same contempt with which they regard politicians. And rightfully so.

CREATION OF A GLOBAL SEA LIFE OCTOPUS WORKING GROUP TO IMPROVE CEPHALOPOD HUSBANDRY

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Introduction

Around the world, cephalopods are a common and popular species in public aquariums. As with all animals kept in aquaria, there is great importance for maintaining high standards of animal welfare of this highly complex group (Hastein, *et al.* 2005). In soft bodied organisms such as cephalopods, the potential behavioural and complex health issues found in these species are often poorly identified (Gestal, *et al.* 2019). This is due to lack of information or expertise on health issues that are common to many species of molluscs. Information from research and aquaculture communities which can contribute to our understanding of these animals is often slow coming into the public aquarium domain (Iglesias, *et al.* 2014). Even in research domains, the welfare of cephalopods and the standardisation of procedures are only now being filtered to the greater research community (Fiorito, *et al.* 2014). Since 2013, cephalopods have had protection under the European Union (EU) legislation in a research setting. At the point of the ratification into each EU state legislation, basic guidelines and husbandry guidance were required, due to member states needing to enforce and licence procedures on cephalopods. As a result, a European Cooperation in Science and Technology (COST) action was funded to bring scientists together to reach a consensus (Fiorito, *et al.* 2014, Fiorito, *et al.* 2015). This workgroup approach of sharing knowledge accelerated the outcomes and standardised the procedures.

Within the accredited zoo and aquarium associations (AZA, BIAZA, EAZA, etc.) work groups are often formed, but typically focus on one or two species; therefore, only creating very specific guidelines for the aquarium industry (i.e. BIAZA GPO manual 2011, AZA GPO manual 2014). In order to promote better sharing of existing data, promote collaboration and identify future research needs, a cephalopod working group was created within the SEA LIFE family, which now contains over 50 aquariums in 17 countries. It was expected that by a formation of a working group, clear actions and standards could be put in place with more stringent oversight and guidance. In addition, new innovations would be formulated as a result of coordinated networking and the various expertise (veterinarians, researchers, aquarists, curators, etc.) within the group. In 2015, the work group conducted a review of all cephalopods and their survival within all SEA LIFE aquariums.

Methods: The Questionnaire

Formation of the SEA LIFE Global Octopus Working Group (GOWG) in 2015 drew in expertise from all levels of the business. It was important to include aquarists, who had daily interactions with the animals, as well as veterinary and curatorial staff, and scientific expertise.

The need to establish baseline data was determined. In August 2016, a questionnaire on cephalopods was sent out to all SEA LIFE sites via the SEA LIFE working group. A Google® form was used for collation of data to allow real time analysis and identify which sites had filled out the questionnaire. This survey was developed to understand current husbandry on all cephalopods within SEA LIFE aquariums, including water quality, nutrition, maintenance, veterinary procedures and treatments, life support and tank designs. In addition, health and pathological data were analysed to produce a health guide for use by both veterinary staff and curatorial teams. This would assist in better understanding of disease processes and their diagnosis. All groups of cephalopods (octopus, cuttlefish, squid, and nautilus) were analysed; however, in this paper we will limit our discussion to octopus. The questionnaire was completed by 45 sites out of 50 (in 2016) within the global group of SEA LIFE aquariums.

Results

There was a 90% completion rate of the questionnaire within the group. The 10% who did not complete in most cases did not currently have octopus. Since a previous questionnaire conducted in 2011, two additional species had been added to our collections with another two removed from our collections (Table 1). *Enteroctopus dofleini* (GPO) had an increased in our collections, most likely due to an increased presence in North America in the five-year period. Of the seven species exhibited at the time, *Octopus vulgaris* and *E. dofleini* were the most prevalent in SEA LIFE aquariums.

Table 1. Comparison of percentage of octopus broken down into species between the two questionnaire years showing an increase in *E. dofleini* and a decrease in *O. vulgaris.*

Common Name	Scientific name	Number 2011	Percentage 2011	Number 2016	Percentage 2016
Common	*O. vulgaris*	21	64	20	51
Giant Pacific (GPO)	*E. dofleini*	7	21	13	33
Red	*O. rubescens*	1	3	0	0
Lesser	*E. cirrhosa*	2	6	2	5
Bimac	*O. bimaculoides*	1	3	0	0
Tetricus	*O. tetricus*	0	0	2	5
Callisoctopus	*C. macropus*	0	0	2	5
Total		32		39	

A specific section was dedicated to *E. dofleini* due to focus of AZA and BIAZA on guidelines in this species of octopus. Variation of the husbandry techniques within the group became evident despite sites following AZA and BIAZA guidelines. Typical cause of death was noted as senescence, which was quickly noted as insufficient due to weight, size and lack of reproductive evidence in a number of cases. It was noted that many facilities never medically treated their GPO's or attempted any regular health physicals. Longevity also varied within and between regions, from 5 months to 3.5 years, which gave a basis for further investigation. It became clear that our aquariums needed further support in order to diagnose and treat diseases and identify negative behavioural trends before serious issues arose. Therefore, a full report was

submitted for consideration to the SEA LIFE welfare department to allow implementation of new protocols.

Analysis of Health Submissions

After it was identified that over 80% of our collection consisted of two main species, a retrospective review of animal health enquiry forms and pathology information submitted by SEA LIFE sites was conducted by our veterinary team. Suspected octopus health issues were submitted by the husbandry teams to the veterinary staff to determine an animal health problem (Figure 1). In the highest amount of cases, 31% of octopuses were identified or described as being inactive. Clear diagnosis of symptoms required veterinary assistance, highlighting the need to provide staff with a better resource to identify health problems. Table 2 shows an example of the diagnostic tool chart developed in order to assist staff in understanding and identifying potential health symptoms, along with their potential diagnosis. The diagnostic tool was built from existing literature with input from the veterinary team. By using both behavioural and physical symptoms, it was considered that there would be a faster diagnosis and therefore effective treatment. With this tool and additional retrospective pathological reviews, we hope to present a future paper of diagnostic cases and outcomes.

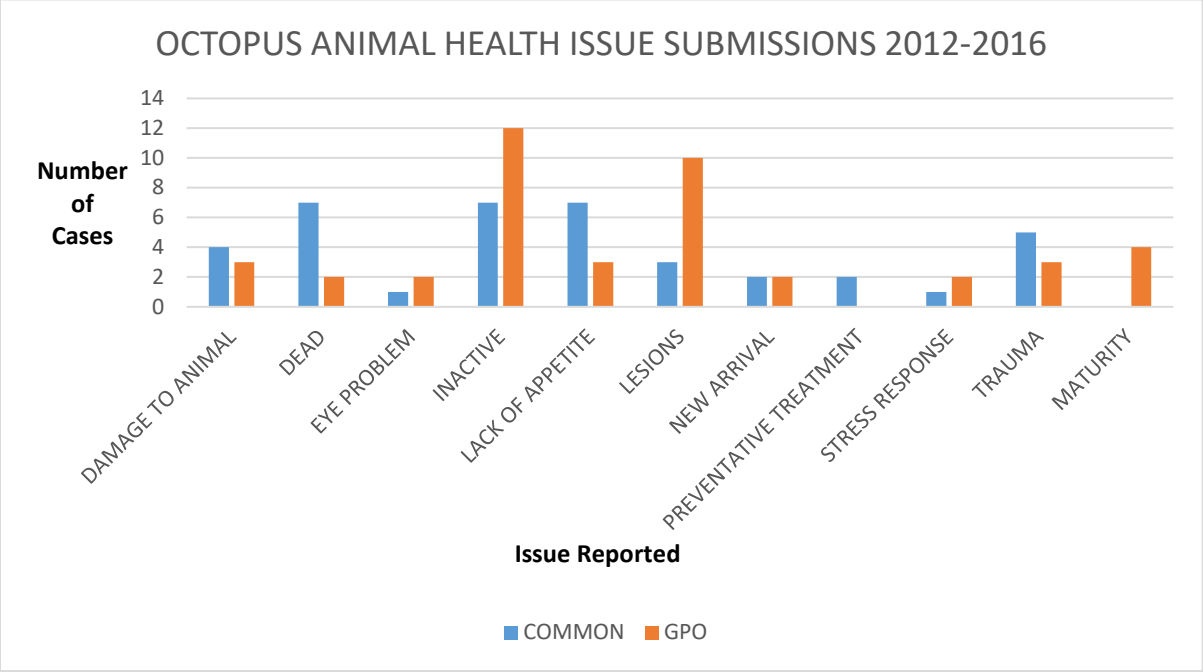

Figure 1. Potential health issues identified by aquarist staff within the period 2012-2016. The largest group for GPO is the inactive health issue which could be signs of lethargy or cases requiring veterinary assistance, as clear diagnosis could not be determined by staff.

Discussion

With the construction of a specialised group, new recommendations and policies were established. A list of group outcomes and actions was constructed for all SEALIFE sites (Table 3). This was compiled from the analysed answers within the questionnaire, which highlighted areas of improvement. It is important that standardisation occurs across the group and that consistently

high standards are maintained. Similar standards were outlined though the AZA GPO manual (2014); however, this only pertains to one species of octopus. In the future there will be closer work with research and aquaculture communities, in order to fill in the gaps of knowledge for animal care and physiology related issues (Fiorito, *et al.* 2014).

Table 2.: An example of a diagnostic tool for aquarium staff with a link to various treatment options to be discussed with a vet. Both physical and behavioural symptoms would be used to help reach a diagnosis.

Symptoms		**Possible Diagnoses**
Behavioural	**Physical**	
Autophagy Lethargy Food Refusal Recently mated or laid eggs	Erosive skin lesions Curly tentacles Buoyancy issues Cloudy eyes Discolouration	Senescence
Jetty Constantly/Uncontrolled jetting Colour flashing Hiding constantly Inking	Mantle swelling Erosive skin lesions Cloudy eyes Tentacle Damage Fluffy skin growths Excessive mucous production Buoyancy issues	Physical trauma
Hiding constantly Food refusal Lethargy Jetty constantly/Uncontrolled jetting	Mantle swelling Erosive skin lesions Cloudy eyes Fluffy skin growths Excessive mucous production Buoyancy issues Discoloration Abnormal discharge from mantle, mouth or eyes	Bacterial infection
Jetting constantly/uncontrolled jetting Food refusal Lethargy Excessive Cleaning	Erosive skin lesions Discolouration Fluffy skin growths Excessive mucous production Tentacle damage Fluid retention, oedema	Parasite infection
Jetting constantly/uncontrolled jetting Food refusal Lethargy	Erosive skin lesions Tissue necrosis Tentacle damage Excessive mucous production Fluid retention, oedema Buoyancy issues Sudden death	Toxicity
Jetting constantly/uncontrolled jetting Food refusal Lethargy Hiding constantly	Erosive skin lesions Tissue necrosis Tentacle damage Excessive mucous production Buoyancy issues	Fungal Infection

Table 3. Outcomes and recommendations from the SEA LIFE OWG as part of company-wide review. Recommendations were to be implemented as soon as possible, with future directions to be worked into 3-5 year plans.

Area of analysis	Recommendations of work group	Future Directions
Life support systems	• Minimum water quality parameters • Minimum equipment requirements • Display design and volume	• Effect of size and exposure of tank analysis • Continuous monitoring of parameters • Addition of equipment to existing systems
Behaviour and Welfare	• Established enrichment plan • Ethogram and behaviour guide	• Sharing of enrichment ideas and plans • Digital recording of behaviour
Health Procedure	▪ Suitable quarantine procedures • Regular weighing and measuring of octopus (monthly) • Use of health chart for diagnosis • Examination and submission of samples for histopathology examination	• Construction of standardised quarantine procedures • Metabolic and consumption rates at varying temperatures • Refinement of health chart used • Review and analysis of pathological data

Our hope is that the findings of our work group have larger implications within the zoo and aquarium community. With a large data set and variation of species, improved standardised care leads to a greater scope for investigation into overriding questions, such as determining age and welfare in our collections. A secondary goal is to make improvements on tank designs and standardized life support and water quality parameters. In addition, by producing tangible outputs we can disseminate information to other interested groups. Findings from the questionnaire are to be cited in the upcoming AZA GPO manual. Health cases are outlined in an upcoming book of cephalopod diseases (Camino, *et al.* 2019) and findings have been presented in the European community at several conferences.

Conclusion

Increased communication and unification are needed in the cephalopod aquarium community to better understand the advanced husbandry requirements of these complex animals. By increasing the knowledge base of cephalopods in our collections, we can have far reaching goals, and can ideally increase longevity and improve welfare of these charismatic species. Our goal is to openly share our shortcomings and identify opportunities to approach the care of cephalopods more effectively. It is hoped that the wider aquarium community will continue to benefit and collaborate with the constructed GOWG to further our understanding of these amazing invertebrates.

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TEN MINUTES TO TRAIN: TARGET TRAINING FRESH WATER STINGRAYS (*Potamotrygon leopoldi* & *P. motoro*)

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Introduction

For decades, animal training through operant conditioning and positive reinforcement has been used in zoos and aquariums to increase the likelihood that an animal offers a requested behavior. Animals learn to perform a behavior, typically by being offered a positive stimulus (something that the animal wants, typically food), called a positive reinforcer or reward (Ramirez 1999). Indeed, training is an important tool for animal care professionals, improving animal care and welfare, and in more recent years has been utilized for a wider range of animal taxa. Training is used to better manage animals (Barth 2003), improve safety (Fleming and Skurski 2014), increase medical care abilities through voluntary veterinary procedures like ultrasounds and exams (Belko et al. 2018, Hellmuth et al. 2012), and enhance guest experiences and education (Anderson et al. 2003) for most groups of animals that are regularly maintained in managed settings. Training for most animal keepers, however, can be a daunting task due to other duties and daily time constraints. Although beneficial, training often becomes secondarily important to the top priorities of general husbandry, animal welfare, and guest experiences rather than being incorporated into these tasks. But training can and should be an integral part of husbandry, welfare and guest experiences.

The Arizona Center for Nature Conservation/Phoenix Zoo houses a polka-dot stingray (*Potamotrygon leopoldi*) and an ocellate stingray (*Potamotrygon motoro*), along with a few other freshwater fish, in a 1300-gallon outdoor tank (Fig. 1). Prior to beginning this study, the four-year-old polka-dot stingray would occasionally take food from a feeding stick whereas the one-year-old ocellate stingray did not. Both stingrays were acclimated to the trainers, but rarely interacted with them in the water. We developed a training plan to target train both freshwater stingrays with a strict focus on time management, adhering to a time allotment of only ten minutes each day for a six-week period. The goal was to demonstrate that fish are capable of learning fairly quickly with only a minimal amount of effort if this effort is consistent.

Materials

- Yellow (with black stripes) target (31 cm x 21 cm; see Fig. 2)
- Blue target (31 cm x 21 cm; see Fig. 2)
- Two feeding sticks
- Training clicker
- Shrimp reward
- Underwater camera
- Timer

Figure 1. The Uco Pond at the Arizona Center for Nature Conservation/Phoenix Zoo. This exhibit houses 1.0 polka-dot stingray (*Potamotrygon leopoldi*) and 1.0 ocellate stingray (*Potamotrygon motoro*), trained in this article, along with 1.0 pacu (*Colossoma macropomum*), 0.0.1 plecostomus (*Hypostomus plecostomus*) and 0.0.3 lungfish (*Lepidosiren paradoxa*).

Methods

We created consistent daily husbandry protocols prior to training implementation. The only change made during the six-week training was the order in which animals were trained (see below). This change was made because the ocellate stingray was not responding to the target when the polka-dot stingray was trained first. We decided that a change in order might provide this animal with a better chance of succeeding. Daily maintenance and training methods were as follows:

- Polka-dot stingray was assigned to the yellow target for all sessions.
- Ocellate stingray was assigned to the blue target for all sessions.
- We kept a consistent morning training time of approximately 0800h.
- The keeper entered the water for each training session.
- The underwater camera was placed at the bottom of pool.
- Three underwater clicks signified the beginning of each session.
- The target was introduced, and the timer was immediately started at the beginning of each session.
- The yellow target (polka-dot stingray) was introduced first for the first two weeks (9 Oct-23 Oct 2018). The blue target (ocellate stingray) was introduced first for the next two weeks

(24 Oct-6 Nov 2018). For the last two weeks, both targets were introduced at the same time (7 Nov-20 Nov 2018).

- Shrimp was used to reinforce successful targeting to the correct target (as defined above).
- If the behavior had not been completed after 10 min, the session was ended.
- General tank maintenance was performed after each training session.
- Daily diet for both animals was received through afternoon scatter feed at approximately 1500h.

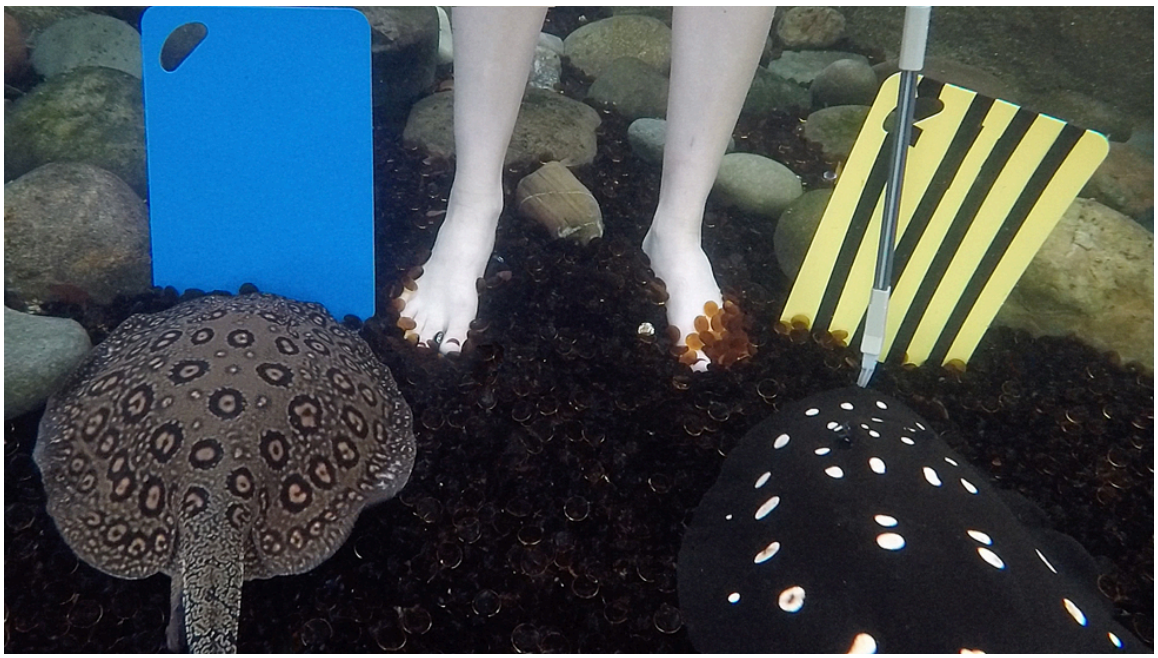

Figure 2. Blue and yellow/black target boards that were used to target train the ocellate stingray and the polka-dot stingray, respectively, at the Arizona Center for Nature Conservation/Phoenix Zoo.

Results

For the first two weeks, the ocellate stingray did not successfully target. During this period, when the yellow target was introduced first, the polka-dot stingray completed the behavior all but one time. When the behavior was completed, time to completing the requested behavior varied from less than one min to just under 7 min (Fig. 3 and Fig. 4).

During weeks three and four, the blue target was introduced (i.e., we attempted training the ocellate stingray) first. During these two weeks, the ocellate stingray did not complete the requested behavior three times and the polka-dot stingray did not complete the requested behavior two times. Both rays improved their training times during these two weeks, and for the last five sessions, both completed the targeting behavior in less than four min (Fig 3 and Fig. 5).

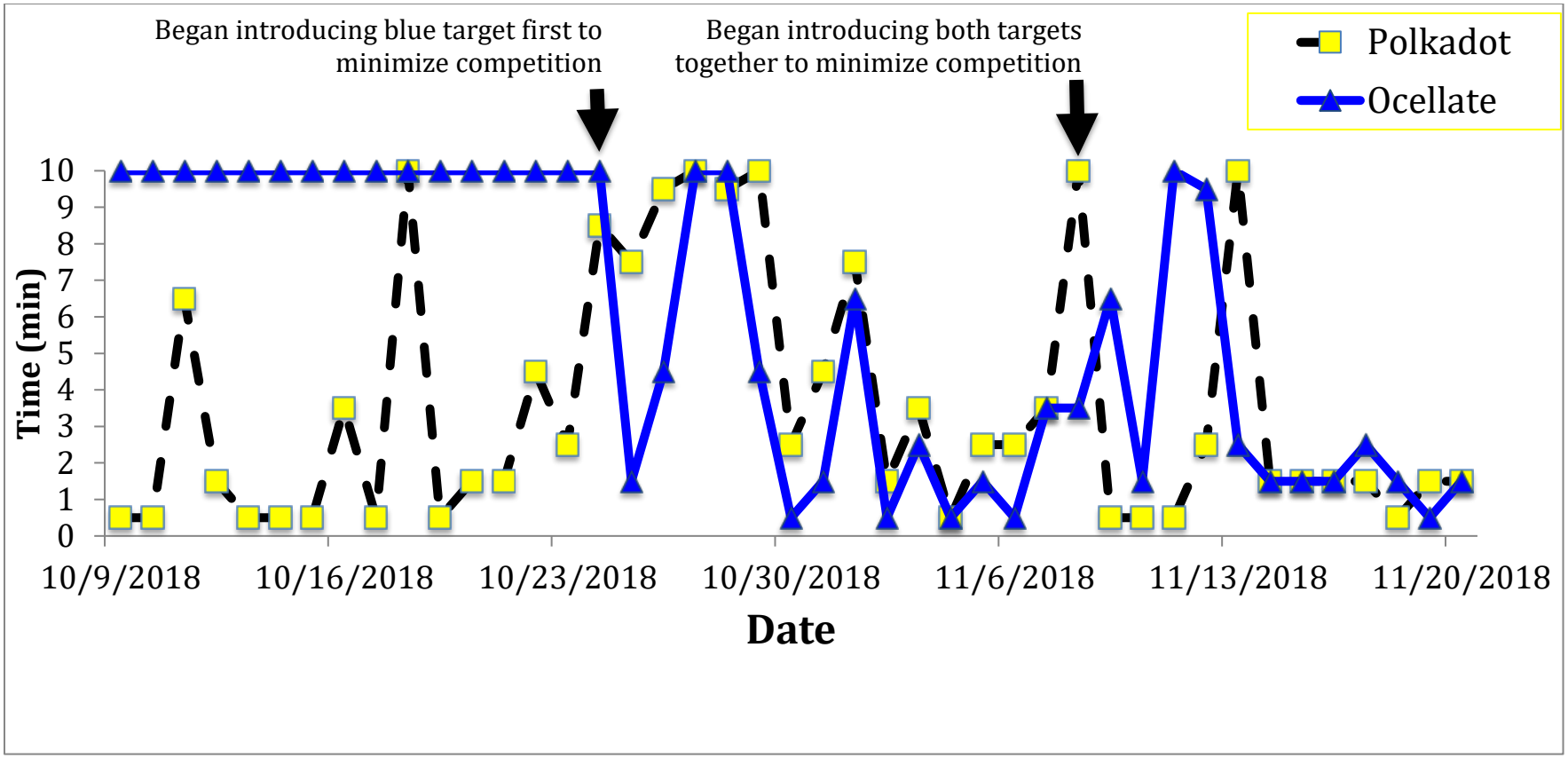

Figure 3. Time to completion of requested targeting behavior for polka-dot stingray (denoted by yellow boxes and black, dashed line) and ocellate stingray (denoted by blue triangles and blue, solid line) during a six-week training course at the Arizona Center for Nature Conservation/Phoenix Zoo between 9 Oct and 20 Nov 2018. If an animal did not successfully target after 10 min, the session was ended (thus the maximum time possible to be recorded is 10 min).

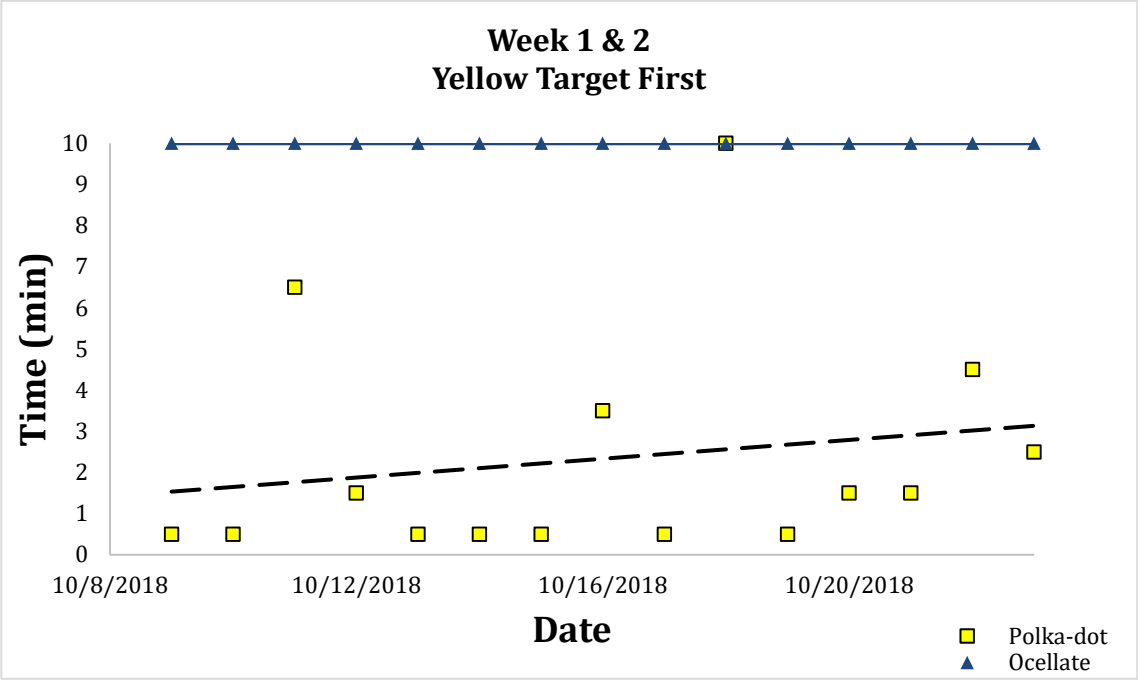

Figure 4. Time to completion of requested targeting behavior for polka-dot stingray (denoted by yellow boxes and black, dashed line) and ocellate stingray (denoted by blue triangles and blue, solid line) during the first two weeks of a six-week training course at the Arizona Center for Nature Conservation/Phoenix Zoo between 9 Oct and 23 Oct 2018 when the yellow target (i.e., that for the polka-dot stingray) was presented first.

During weeks five and six, both targets were introduced at the same time. The ocellate stingray did not complete the behavior one time, and the polka-dot stingray did not complete the behavior two times. Again, both rays improved their time during this two-week timespan and in week six, both rays completed the targeting behavior in less than three min each session (Fig. 3 and Fig. 6).

Discussion

When the yellow target was introduced first during weeks one and two, competition was extremely high from the ocellate stingray. Once the polka-dot stingray achieved the reward, the ocellate stingray shifted attention away from the target and towards the polka-dot stingray. Motivation toward the targets for both stingrays was highest at the beginning of each session. For this reason, we decided to alter the order of training and offer the blue target for the ocellate stingray first.

During weeks three and four, when the blue target was introduced first, performance and competition changed. Although it initially took the polka-dot ray longer to complete the behavior, the ocellate stingray became successful at targeting and both animals improved their time to

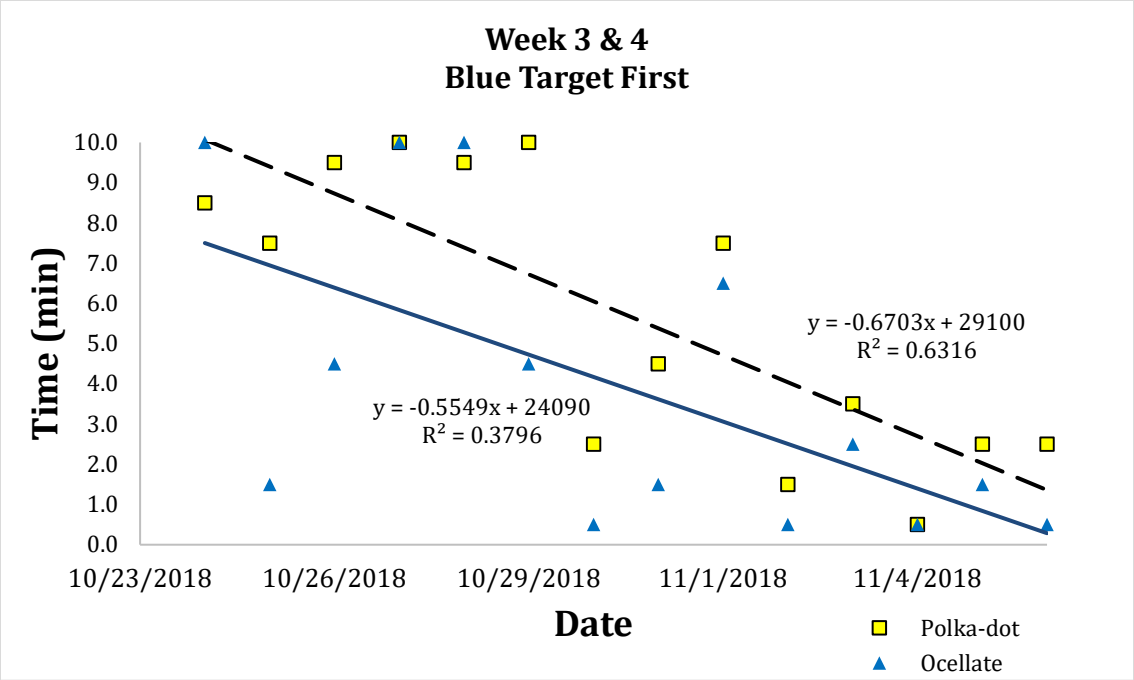

Figure 5. Time to completion of requested targeting behavior for polka-dot stingray (denoted by yellow boxes and black, dashed line) and ocellate stingray (denoted by blue triangles and blue, solid line) during the weeks three and four of a six-week training course at the Arizona Center for Nature Conservation/ Phoenix Zoo between 24 Oct and 6 Nov 2018 when the blue target (i.e., that for the ocellate stingray) was presented first.

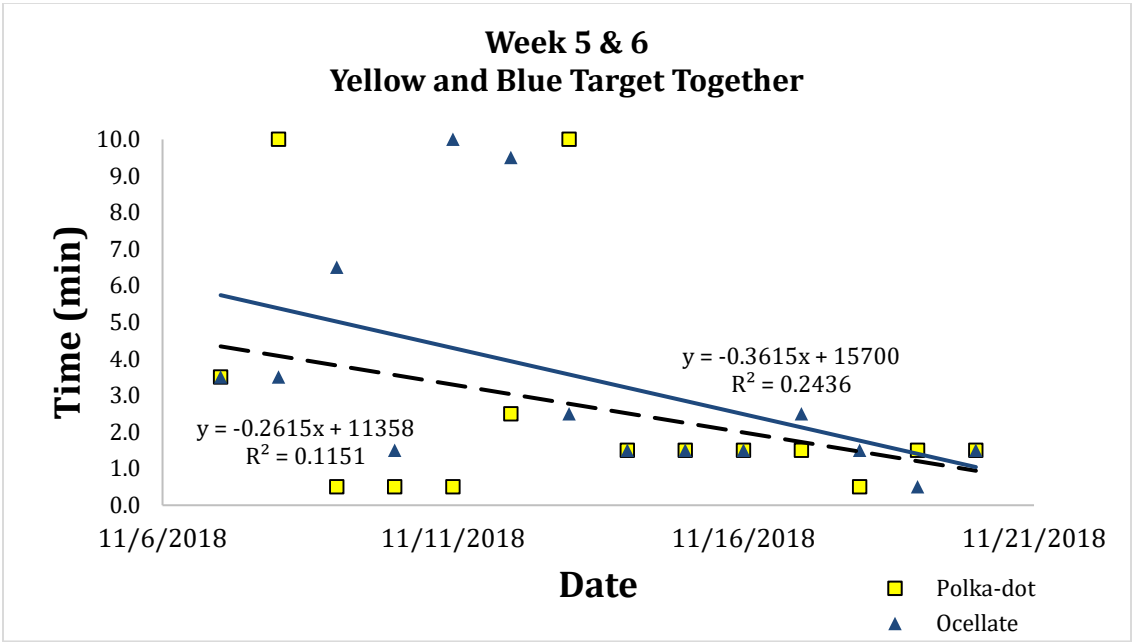

Figure 6. Time to completion of requested targeting behavior for polka-dot stingray (denoted by yellow boxes and black, dashed line) and ocellate stingray (denoted by blue triangles and blue, solid line) during the weeks five and six of a six-week training course at the Arizona Center for Nature Conservation/ Phoenix Zoo between 7 Nov and 20 Nov 2018 when both targets were presented simultaneously.

complete the requested behavior after one week (Fig. 5). That said, the polka-dot stingray began to compete. Overall the polka-dot stingray was still successfully targeting, but the delay of his target entering the water, at least initially seemed confusing as it caused him to turn his attention to the ocellate stingray. It is interesting to note the drastic decrease in the time to successfully target after about one week (Fig. 3), however, indicative of learning. Because of the competitive behavior observed in the polka-dot stingray, we decided it would be best to introduce both targets simultaneously to try to minimize competition between the animals and control feeding.

At weeks five and six both targets entered the water simultaneously, giving each stingray an equal opportunity to approach their correct target at the start of each session. Competition was reduced, if not eliminated, as both animals quickly approached the targets and received food rewards. Again, although we initially saw animals take longer to successfully target or fail to do so altogether in week five, by week six, they were targeting very quickly to the correct target (Fig. 6), demonstrating the ability to rapidly learn in both species.

Through this consistent six-week trial, we were able to demonstrate the ability to quickly train target behaviors in both the polka-dot stingray and the ocellate stingray with minimal time commitment by animal care staff. As we move forward, our plan is to continue to use target behaviors, introducing both target boards into the water at the same time, to eliminate competition between these animals and control feeding, which might prove useful for future medical treatments as well.

Conclusion

In order to provide better care for animals in managed settings, it is beneficial for staff at zoos and aquariums to invest time in training. Target training can assist in distributing food, reducing competition, and increasing animal participation in medical examinations. In this six-week long training exercise, we were able to document learning in our ocellate stingray and polka-dot stingray, as both animals dramatically improved (i.e., decreased) their time to successfully target (Fig. 3, Fig. 5 and Fig. 6). By doing so, food competition between animals was also decreased, and we were able to ensure that both animals received an equal amount of food. By devoting only ten minutes a day for six weeks, both rays were able to learn to pole feed and target train, including target differentiation. We were able to demonstrate that time commitments for training need not be immense. Those in the animal care profession should not be intimidated by the thought of animal training and the associated time commitment, but instead think critically about how to use training to improve animal care and efficiency. Just by starting small and being consistent, animal care staff can get their feet wet in the training pool to see where the road towards improving animal care leads.

Acknowledgements

We are grateful to a number of Phoenix Zoo staff for guidance, support and revising of this manuscript.

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Never Have I Ever, Aquarium Edition

C.M Schwimm

Give yourself one point for each item NOT done, lowest score wins! I scored zero – how about you?

- Worked a 12-hour shift in soaking wet shoes
- Secretly thought Glo-fish were cool
- Forgotten to glue a PVC joint
- Ate lunch while prepping food
- "Shook hands" with an octopus
- Participated in a fish transport longer than 36 hours with no sleep
- Tube-fed a fish
- Used ZIMS or TRACKS
- Started a siphon by mouth
- Pulled regurgitated food from tanks while hungover (and kept your lunch down)
- Raised a batch of clownfish
- Been bitten by a shark (*Chiloscyllium* counts)
- Had a person on a bus complain "you smell like dead fish"
- Tested positive for *Mycobacterium marinum*
- Sniffed some MS-222 "just to see"
- Been stung by a lionfish
- Spoke at a RAW or NAW meeting
- Have reoccurring lucid dreams about collecting fish on a reef
- Microwaved some "extra" shrimp as an afternoon snack

HOW TO BECOME THE LIFE OF THE PARTY YOU WEREN'T INVITED TO: A GUIDE TO GETTING INVOLVED IN AZA PROGRAMS

Kelli Cadenas, Curator kelli.cadenas@merlinentertainments.biz

SEA LIFE Michigan, 4316 Baldwin Road, Auburn Hills, MI, USA

The Challenge of Getting Involved

Do you want to be a leader in the AZA aquarium community? I did, and I launched myself on that path in 2014 when I went to my first RAW TAG day. At that point I had been an aquarist for five years at National Aquarium and was pretty sure I had gotten the whole Aquarium Industry thing figured out. I was already on the AquaticInfo listserve, I knew what SECORE was and had heard of Project Piaba, so I was feeling pretty on top of things. I was able to go to my first RAW (Regional Aquatics Workshop) because I had a presentation accepted and I had no clue what to expect. The TAG meetings have always been held the day before RAW officially starts, so most RAW attendees don't actually get to go and like me, a lot of them don't know what "TAG" actually means anyway. My mentor at the time, Brian Nelson, very patiently explained what all the acronyms stood for and I was absolutely amazed at all the conservation work and animal programs presented during that day full of meetings. I knew then that I wanted to be a part of that. Then… reality. I was an aquarist, and a busy one. My institution already had their own AZA goals and Institutional Representatives (IRs) assigned for the various TAGs and those people were also the IRs for the studbooks and Species Survival Plans (SSPs.) I took care of the freshwater rays, but the Freshwater TAG IR was in another department, and although I sent an email and declared myself the IR for the Potamotrygon SSPs, I have learned that there is a lot more to it that saying it quietly to yourself at your desk. Looking back, I didn't know who the Institutional Liaison (IL) was at the National Aquarium at that time, and still don't. I didn't ask, because I didn't know it existed and that every AZA accredited institution has one. I didn't know that the IL's are in charge of delegating the IRs. I didn't know anything.

Now I'm a curator who has been through an AZA accreditation, am an Institutional Liaison and can make myself the IR of whatever I please. I now manage a team of aquarists and I am not much more successful at getting them involved than I was for myself. I was sure I would be able to navigate all this much easier than my past managers, but I have encountered a whole new world of roadblocks I didn't understand as an aquarist. I have a Senior Aquarist who put together an entire session on invertebrate welfare at the 2018 AZA annual conference. I have an aquarist who has been the main driver of our new sturgeon conservation projects, and when I talk to them about getting involved in AZA programs like the Aquatic Invertebrate Taxon Advisory Group (AITAG) or the Freshwater Fish Taxon Advisory Group (FFTAG) I'm about as useful as I was back in 2014. So, I've decided to find some answers.

What Kinds of AZA Programs Are There?

AZA animal programs are broken up by taxa, but while there are Taxon Advisory Groups (TAGs) dedicated to smaller groupings of species like penguins and rhinoceros, the aquatic TAGs are much broader and include a huge number of species. There are three aquatic TAGs: Marine Fishes, Freshwater Fishes, and Aquatic Invertebrates. These TAGs manage animal programs like studbooks and Species Survival Plans (SSP). Studbook keepers keep track all the individuals of a

certain species held by zoos and aquariums, and recommend animal transfers between institutions to maximize genetic diversity within collections. Some SSPs, like Sawfish, keep track of individuals, but focus more on conservation and education than breeding success.

New programs like AZA SAFE (Saving Animals From Extinction) are creating new opportunities to work on projects like the International Elasmobranch Census (#chondrocensus) the Elasmobranch Blood Project, and the Elasmobranch Animal Care Manual project. There are also new SAFE studbooks that are looking for leadership. The Atlantic Acropora Coral SAFE program and the AITAGs Florida Reef Tract Rescue project are creating opportunities for real world coral conservation.

Set Your Goals, Find Your Passion

Do you want to help out on an already established project or program? Do you have a passion for a specific species (sea slugs maybe?) and want to start your own project or program? Is your institution already doing amazing work and you just want it reported to the appropriate TAG? Knowing what you are passionate about is the first step. Next, you need to look at your institution as a whole, and find out how your project would benefit it. What is your institutions mission? What are their priorities? All AZA facilities with animals that have a studbook and SSP are required to report the ongoing status of those animals and participate in the programs. Do you have a passion for sea horses? You could ask your MFTAG IR if you could step in as the IR for the lined seahorse or big bellied seahorse SSPs. This would mean being the person that always makes sure your sea horse populations are up to date in the studbook, which would benefit your institution as well as helping you get a foot in the door of an existing animal program.

Find Your Institution's AZA Representatives

The next step in your quest to get involved should be to find out who the Institutional Representative (IR) is for the program you want to work in. Your institution should have an IR for all three aquatic TAGS. These might all be the same person, or it could be three different people. You may find that there is someone who is already involved in the TAG and up to date on what's going on, or you may find there isn't a designated IR at your institution, or it may be someone who isn't active in the TAG. Either way, the IR is truly the representative for your institution. They receive updates from the TAG leadership and calls for participation. If you have asked everyone in your husbandry department and can't figure out who your IR is, you can ask your Institutional Liaison (IL.) Every AZA accredited facility has an Institutional Liaison who is responsible for delegating the IR roles, as well as being the main point of communication between the facility and AZA. You may find that you currently don't have an IR delegated, or the person delegated has left the company. ILs have rights to update your institution's IR information on AZA.org.

Get Involved

When asked how she got involved in the MFTAG, MFTAG Vice-Chair Paula Carlson said "I saw a need and raised my hand," which led to her being the TAG secretary for close to ten years. Although she is fully entrenched in the TAG, her passion for sawfish conservation has helped push amazing and impactful initiatives like International Sawfish Day.

Barrett Christie's road to TAG leadership was a bit different, starting first on local conservation projects, including doing freshwater mussel surveys on his weekends, getting to go

on the first SECORE trip because they needed a scientific diver, and eventually six years later, he attended an AITAG meeting where they were asking for help with the first ever AZA AITAG space survey. Now, as a vice-chair of the AITAG with a lot of involvement in programs, Barrett considers a lot of what he does as volunteering, knowing he can't make time for it all with a busy day job. This seems to be a pretty consistent theme among leadership in the TAGs. Brian Nelson, AITAG Chair, has a similar strategy for making time to be involved, saying that while conference calls often fall within his work day, the bulk of the work he does for the TAG is done from home, on top of his work with AquaticInfo.

Is that acceptable for you? AZA is a non-profit organization that is "dedicated to the advancement of zoos and aquariums in the areas of conservation, education, science, and recreation." Volunteering for a non-profit seems like a pretty noble thing to do. But can you do so legally? Are you hourly? Salary? Does it matter? That sounds like a question for your HR department, but it's a question that may help you better understand what working with these projects will look like, and what you really have time for. One of the first things you will find when you apply to get more deeply involved in an AZA program beyond the IR level, is that institutional approval is required. Program leaders and officers will likely need a letter of commitment signed by your institution stating that they will give you the time and resources you need to work on those projects.

Conclusion

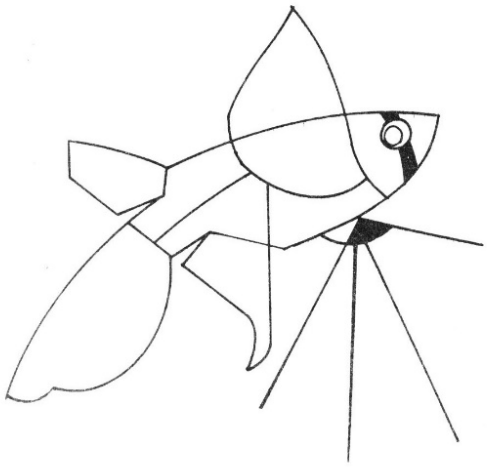
Don't get discouraged. If you don't hear back right away from TAG leadership, know that they have busy jobs too. Embrace your strengths and try to make yourself valuable to these programs. Talk to your manager and find out what your institution is already doing. Go to RAW if you can, and if you can't, find out who from your institution is going and tell them to report back on the TAG meetings! Keep pushing, raise your hand, and call me if you want to write an animal care manual.

Appendix - Acronyms to Know:

ACM – (AZA-approved) Animal Care Manual
AITAG – Aquatic Invertebrate Taxon Advisory Group
ARCS – Annual Report on Conservation and Science
AZA – Association of Zoos and Aquariums
CAP – Conservation Action Program (a historical program type)
CGF – Conservation Grants Fund (formerly CEF)
FCC – Field Conservation Committee
FFTAG – Freshwater Fishes Taxon Advisory Group
IL – Institutional Liaison
IR – Institutional Representative
MFTAG – Marine Fishes Taxon Advisory Group
PMC – Population Management Center (Genetics guidance to SSPs)
RAW – Regional Aquatics Workshop
RMC – Reproductive Management Center (Guidance on contraception, infertility, etc.)
SAFE – Saving Animals From Extinction (many programs for various taxa)
SAG – Scientific Advisory Group

SECORE – Sexual Coral Reproduction (not itself an AZA program, but linked to AITAG and Coral SAFE)
SSP – Species Survival Plan
TAG – Taxon Advisory Group (Manages SSPs)
WAZA – World Association of Zoos and Aquariums
WCMC – Wildlife Conservation and Management Committee (Manages TAGs)

Special thanks to Beth Firchau, Brian Nelson, Barrett Christie, Paula Carlson, and Skylar Snowden for taking the time to answer my questions.



MOVEMENT AND TRANSPORT OF 200+ KG GREY NURSE SHARKS (*Carcharias taurus*)…WHAT COULD GO WRONG?

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Abstract

At the end of 2017 Merlin Entertainments, decided to close the 53-year-old Manly SEA LIFE Sanctuary in Sydney Australia. Engineering reports indicated a rebuild was needed due to structures beyond repair. Home to over 1000 different animals, careful planning was required for their relocation. Six of the fish were 25 to 40-year-old Grey Nurse Sharks (GNS) aka Sand Tiger Sharks (*Carcharias taurus*). These sharks were all in excess of 3.5 meters (11.5 feet) and 200+ kg. The species is no longer able to be obtained from the wild in accordance with the Australian Department of the Environment Recovery Plan for GNS. The movement of the GNS required the development of systems and equipment that would allow the sharks to remain submerged and supported by water through all transfers and avoid traditional capture stress issues. Through careful planning, many long days and nights, and 17 interstate transports each over 1200 kilometers, Merlin Entertainments successfully relocated over 1000 animals including the six aging GNS with zero transport-related mortalities.

Introduction

Manly SEA LIFE Sanctuary was a ground-breaking aquarium over its 52 years of operation. The aquarium opened in 1965 as Marineland. It was considered an eyesore of an attraction according to the Daily Telegraph but was known for being the largest aquarium in the southern hemisphere. In 1989 the aquarium was refurbished and reopened as Underwater World. A few years later in 1992 Coral World International purchased the aquarium and re-opened it as Oceanworld. During this time major renovations included the installation of the longest aquarium tunnel at the time measuring 110m (361ft) in Shark Harbour, the new 4,000,000-liter (1,100,000 gal) tank built under the existing facility. In 1999 Sydney Attractions Group (operated as a branch of the Sydney Aquarium) purchased the aquarium then sold it to Village Roadshow Theme Parks (Sea World Gold Coast) in February of 2008. In December 2010 Merlin Entertainments acquired Sydney Attractions Group officially launching the Manly SEA LIFE Sanctuary in June 2012 with the introduction of Penguin Cove Exhibit (Little Blues). Unfortunately, time took its toll on the aquarium. In January 2018, Merlin Entertainments officially closed Manly SEA LIFE Sanctuary due to engineering reports requiring extensive repair that would require the closing main tank built beneath the original structure.

The exploration of closing Manly SEA LIFE Sanctuary (Manly) aquarium commenced in late 2016. Teams began evaluating the collection and brainstorming methods for the movement of the animals. There were many considerations that needed reviewing such as licensing and permit restrictions of the collection, Australian Protected Species, ability to export but most

importantly staff safety and the animal health and welfare. The biggest question - who has moved a 3.5 metre, 250 kilogram, 40-year-old Grey Nurse Shark? Immediately the naysayers began stating that the closure of Manly would result in the killing of the iconic GNS.

Research began by consulting with those who move sharks routinely across the globe. What would be the cost for them to move the sharks? What is the staff knowledge to conduct the relocation in house? What equipment is available in Australia?

Next was the method of transport. A road transport to the new location was approximately 1,000 kilometers (620 miles) which would take about 14 hours door to door not including loading and unloading. An air transport was slightly less but potentially meant at least six hours of un-monitored animals. Railroad and barge transports were also evaluated. Railroad proving the riskiest and would require a specialized container for loading on a train. The barge system was safe, could haul a greater load capacity, provided more swimming room and access to clean seawater for consistent water changes, and could be performed as all facilities had open access. Unfortunately, this method would take days to complete the transport opening up potential other issues such as weather. For these reasons and after extensive research, a road transport was determined to be the safest method for the animal's welfare.

It was also decided early in the development process that all GNS would not be lifted dry. This was to eliminate any possible trauma or damage. Hence, at all stages of the transport design a wet lift was needed. Avoiding capture and transport myopathy was considered critical.

Design Process

To conduct a road transport of 14 hours, not including loading and unloading time, required careful planning to maintain water quality, animal's ability to move, prevent slosh while not exceeding road weight restrictions. Basics began with water volume, current requirements, turn over time, water movement/slosh, and water temperature. Based on this we calculated it was necessary to degas CO_2, add O_2, remove organics, maintain water chemistry and remove angles in the transport container.

With the needs of the animals understood, what are the needs to insure the transports would be successful? Animals die when they are not being monitored and lacking the ability to intervene if needed. Therefore, we needed the ability to see the animals and monitor water quality at all times.

Shark Transport Design (numbering corresponds with labels in Figure 1)

1. 100mm fiberglass insulated transport tank (2.35 m width x 6 m × 1.1 m high, ≈15 m3) with thick fiberglass insulated lid bolted to a 30 mm gasket. Tank and primary lid can be one piece if easier and needed for additional support. Steel support beams for tank.
    a. Secondary lid needs to be sized to fit shark transport bag and two divers for releasing and removal of bag.
        i. Coverable viewing panels needed in secondary lid. Ideally with ability install LED lighting for visual.
    b. Tank needs ability to be overfilled using a top hat configuration.
    c. Tank lid need access for water quality testing.

2. Filter unit. Contains one filter floss, activated carbon and biomedia for gas exchange
3. Diesel generator(s) mounted below the truck. Back up12 V dry-cell sealed battery, wired to bilge pump.
4. 4x Coverable clear acrylic viewing panels measuring 1000 × 700 (two on each side of the tank.
5. Submersible pump resting in pump pit capable of flowing water up through degas tower and alternate current (left and right side of transport).
    a. Return pips on both lengths of tank to allow for current of 1.3m/s
    b. Submersible pump needs to pump from pump pit to return pipes. Plumbing may be built in to top of tank or internally.
6. Ceramic or stainless steel airstone fed by airline connected to pressurized oxygen cylinder.
    a. Oxygen/airlines need water resistance access.
    b. Oxygen diffused in from of submersible pump for circulation distribution.
    c. Aerators only to be used in emergency situations and will be run from generator.
7. Filter inlet, i.e., PVC elbow mounted through fiberglass insulated lid, connected to bilge pump through 50mm reinforced hose or pipe.
8. Filter outlet, i.e., PVC elbow mounted through wooden lid, returns filtered water above surface of water inside tank.

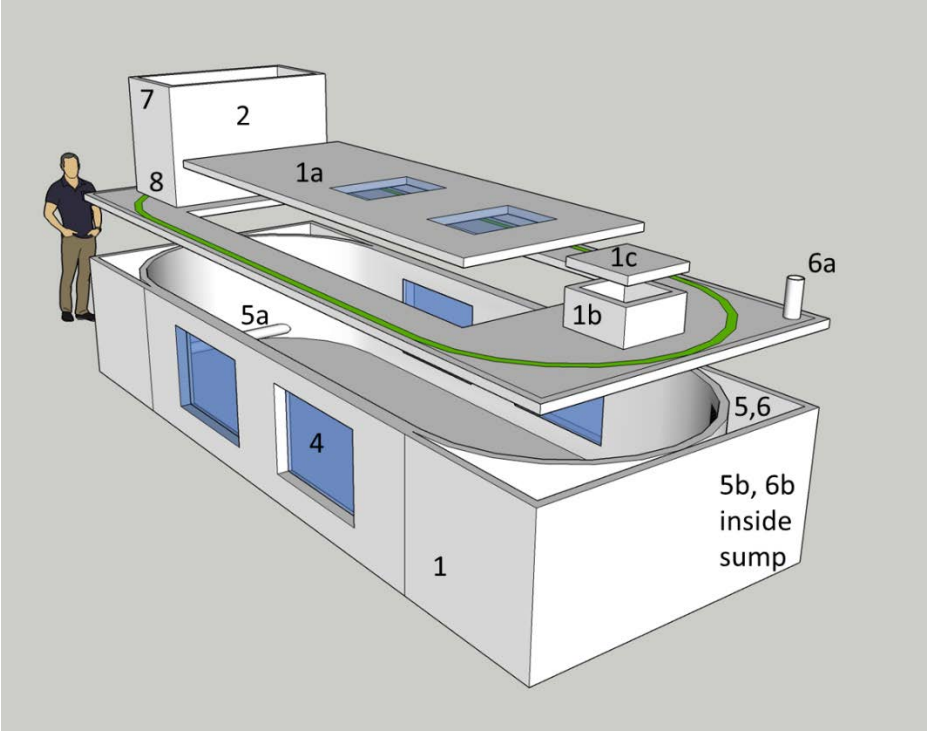


Figure 1. Transport Design (labels explained in text above).

Build

The design was submitted to Advanced Aquarium Technologies (AAT) for engineering and building. The transport container (Figure 2) included a drone camera system allowing for the animals to be seen at all times via Wi-Fi. An Oxyguard Pacific PLC system was used to remotely monitor (worldwide) DO, Temp and pH. The Seneye Home Aquarium monitor system was to be

added for the continuous monitoring of NH_3 (Free Ammonia) and pH. Tank LED lighting was remotely controlled allowing staff to turn lighting on and off as needed.

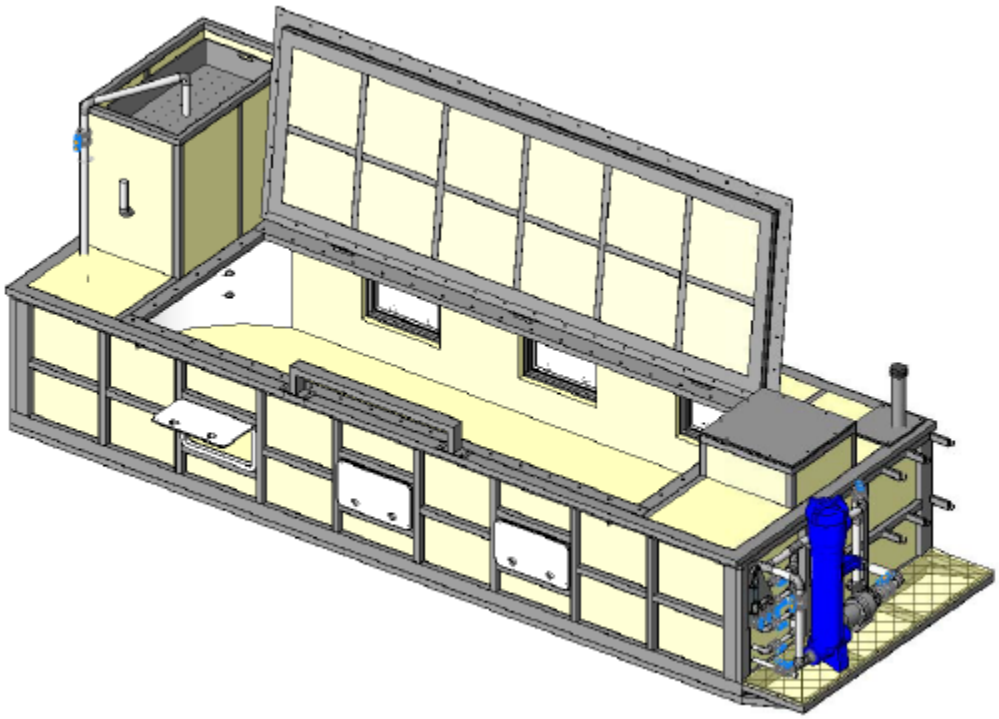


Figure 2. Transport Drawing.

The transport consisted of a water volume of 15,000 liters (3,962 gallons) with a large space for animals to swim and turn, with minimal cross current for oxygen exchange across gills. It was insulated to minimize the water temperature from changing drastically. There was 3.9 x 1.3 m opening allowing for wet lifts of animals preventing the need to remove a 3.5 m, 250 kg animal from the water. The opening could then be sealed with pressurized lid to allow overfilling of the transport therefore preventing water sloshing during the road transport. The transport utilized a built-in sump for water quality monitoring and pump suction. A 1.2 meter degas tower allowed for gas exchange to remove CO_2 with the ability to add blower for increase CO_2 stripping if needed. Oxygen was introduction via a ceramic air stone at the pump intake. The transport would be overfilled to prevent the sloshing of water and hammer of animals against the side walls. Life support system consisted of a small mag drive pump capable of a one-hour turnover time with an attached sock filter for the removal of organics or addition of carbon if needed. The whole system run by a Kubota® Lowboy generator designed for running length periods of time. The transport build provided the ability to make continuous runs (6+ hours) at a time without stopping.

Methodology

The transport was loaded onto the soft sided refrigerated semi-trailer (Figure 3). The transport was placed on rubber pads and secured using straps attached to hold fasts on the sides of the tank. Loading of the transport could be performed using a forklift from topside removal handles or by a crane by converting removable handles to eyelets. The diesel generator was

mounted below the trailer allowing the driver to fill it on fuel stops and insuring exhaust was vented outside the trailer.

Figure 3. Transport loaded on the truck.

Sharks were moved approximately every two days allowing for the load and transport of the shark followed by the return of the transport. Loading and unloading of the sharks took approximately 30 to 60 minutes from tank to the transport container. The transport of the shark commenced by lifting the shark upwards and out through an access hatch cut in the ground above the med pool at the Manly SEA LIFE Sanctuary. The shark was then moved hundreds of meters (including up a steep grade) to the transport truck, where the stretcher was then be lowered into the transport, with the added caveat of avoiding underground storage and seawalls (Figure 4).

The sharks were in a sectioned off area of the large Oceanarium and fasted for four to five days before the transport. The selected GNS was then swum into the med pool in a gentle controlled manner. During this same time the transport was filled with system water in order to be ready to receive the animal. Once in the med pool, a member of The Aquarium Vet team observed the shark before deciding to mildly sedate using Phenoxyethanol at a rate of 0.15 to 0.20 ml per litre of water. Phenoxyethanol is used as a preservative in cosmetic products and also as a stabilizer in perfumes and soaps. It was commonly used in aquaculture in the 1960s. Phenoxyethanol was chosen for a couple reasons, (1) Tricaine methanesulfonate is considered an illegal substance therefore virtually unavailable in Australia and (2) the animals' ability to recover quickly once in clean water.

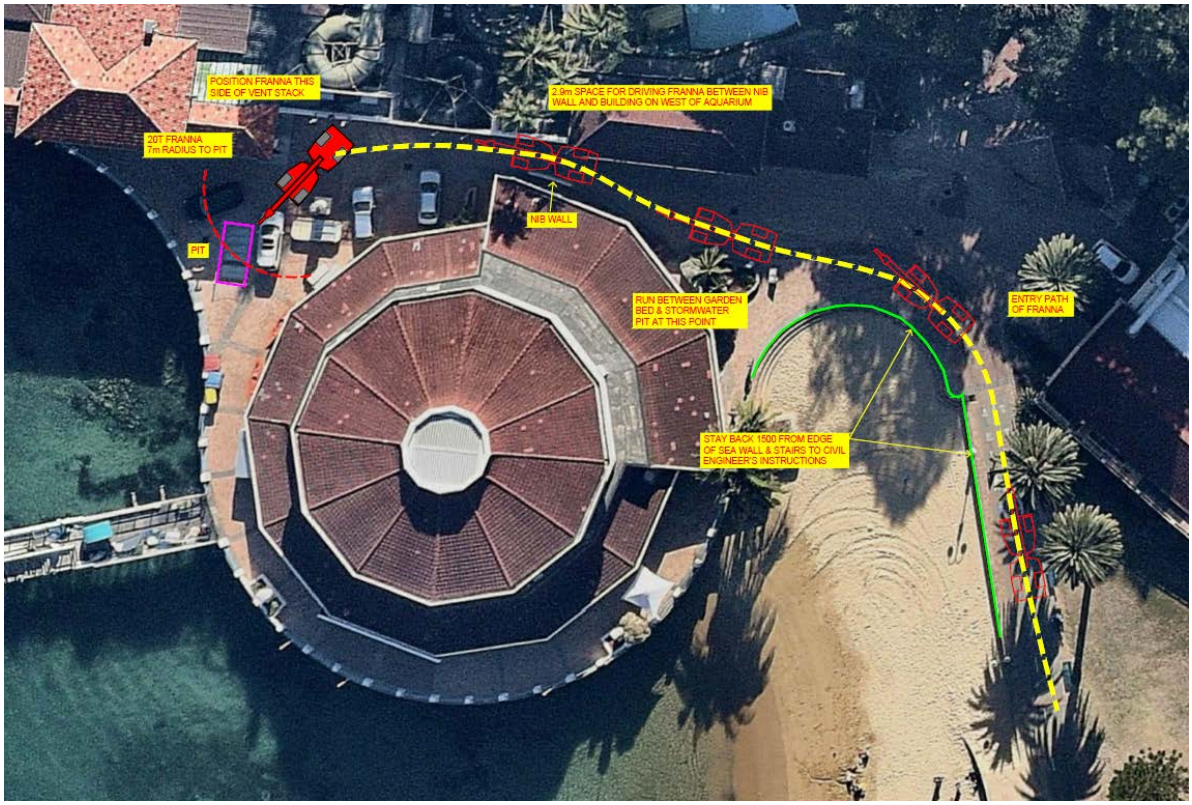


Figure 4.– Arial view of Manly loading route and requirements.

Sedation with the Phenoxyethanol took about 20 to 35 minutes, by which stage the shark had almost stopped swimming. A team of divers then gently restrained the shark. Due to the sedation, tonic immobility was not required or used at any stage of all the transports. The veterinarian next collected a blood sample via a lateral approach in the tail. Blood was collected pre- and post-transport for all GNS both for Abbott i-STAT (Handheld Point of Care Analyzer) assessment of blood pH and gasses (CG4+ and CHEM8+ cartridges) as well as a full profile run at a local veterinary laboratory as baseline values for future use.

After collecting the blood sample, each GNS had the following prophylactic medications administered by intra-muscular injection by the veterinarian:

- Dexamethasone sodium phosphate (cortocosteroid)
- Florfenicol (Nuflor LA - antibiotic)
- Vitamin C

Once the animal was sedated, a pipe was passed into the stomach and it was burped. This was to remove all air from the stomach to allow the animal to settle during the transport. Following this the shark was then maneuvered into the shark transport bag (Figure 5).

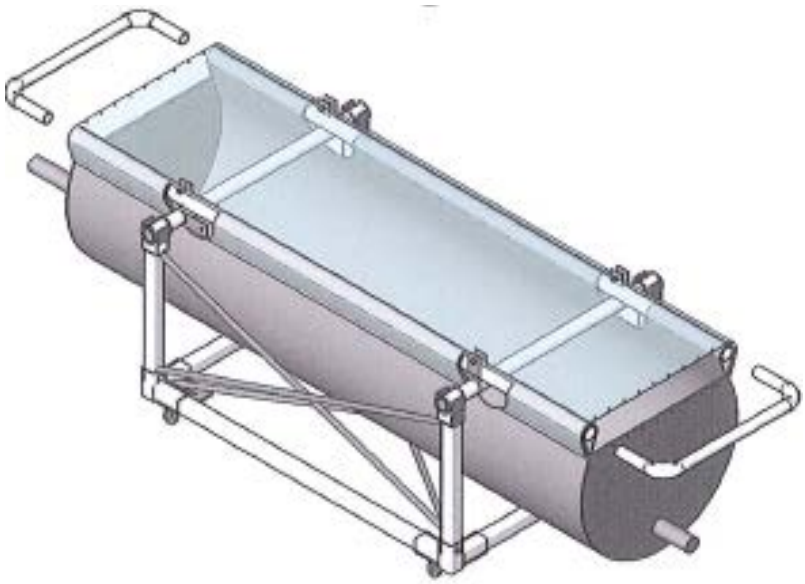


Figure 5. Shark Bag.

The shark transport bag is constructed from Hypalon fabric (used to build Zodiac inflatable boats). This fabric is designed for allowing punctures without tearing. A shark tooth puncturing the bag would simply result in a small leak of water. This occurred once during a load and was easily mended with peppermint chewing gum. The bag was 3.5 m long and 0.95 m wide. The bag was support by a steel frame with cross members support to insure the bag maintained its shape. The cross members where designed to be easily installed once the shark was in the bag. The volume of the shark bag was 3000 litres of water. The key use of the shark bag was to avoid any dry lifts of the large animals that would have most surely resulted in fatal injuries.

The bag was then covered and lifted out of the hold using a Franna® crane (Figure 6) to commence the movement through the obstacle course (Figure 3) to the preloading position.

Oxygen was maintained at about 150% during the entire loading and transport. Due to the clearances of the top the transport to the ceiling of the trailer the Franna crane could not be used for lowering the shark bag into the transport. Hence, at the preloading position the shark bag with the shark was transferred from the Franna Crane to a Telescopic Handler Forklift (telehandler). See Figure 7.

At this time a 100% water change was performed maintaining DO at 150%. Once the exchange of the bag from the crane to the forklift was completed the final phase of loading into the truck commenced. The telehandler was then used to load the shark (inside the shark bag) into the transport tank (Figures 8 and 9). Once in the transport container, the shark was moved from the stretcher. The stretcher was then removed for the actual transport and stored on the truck for the transport.

Figure 6. Lifting the stretcher through the hole cut above the med pool.

Once the shark was in the transport container, the veterinarian and staff monitored the shark and water quality for approximately an hour while performing another 100% water change. Construction equipment remained on standby if unloading was required due to an emergency. Once staff were satisfied with the animal's behavior and water parameters, the transport container was sealed to begin the 1,000+ km road trip to its new facility.

Figure 7. Transferring from the Franna Crane (right) to Telescopic Handler Forklift (left).

Two aquarists and one veterinarian followed the transport in a chase car while visually monitoring the shark via the drone cameras and water parameters via an APP on their telephones. Ranges for the monitoring easily was maintained hundreds of meters behind the transport truck.

Transport runs required stopping for fueling only and were able to complete a minimum of six hour runs with no concern for animal welfare. Respiration rates were regularly monitored via the camera system. It was not required, at any stage during any of the transports (14 hours plus) to open the transport container.

On arrival, the transport container was opened and an acclimation process commenced. However, during the transport because of the close monitoring and control it was possible to alter the temperature as required and prevent the pH from dropping and so acclimation time was minimal.

Once acclimated, another dose of phenoxyethanol was added to sedate the shark. When sedated, another blood sample was collected, similar to before the transport. The stretcher was placed inside the transport container, the shark eased into the stretcher and with a forklift it was lifted out and again moved from the crane to a forklift telehandler and on into the holding pool of their new Oceanarium. Recovery from the phenoxyethanol was very rapid and within 30 minutes all sharks were swimming as though they had not been transported.

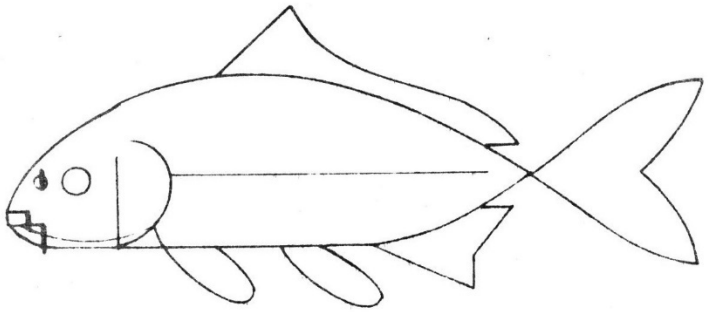
Figures 8 & 9. Loading the stretcher (with shark inside) into the shark transport container.

The use of the i-STAT machine meant that blood gas and pH values were available in real time. This was to ensure that capture myopathy was avoided or if it occurred was detected early. All samples were within normal ranges both before and at the end of the transports. The use of the phenoxyethanol certainly aided the smooth handling of these large mature GNS at the critical stages of loading and unloading, leaving them to swim unsedated for the 12 to 14-hour journey.

Conclusion

Overall 17 interstate trips averaging 1000km one way were conducted to move all the animals from Manly. Minor issues did occur but were quickly resolved. Issues included calibration of probes on the trial transport to test functionality. Staff was requested to calibrate probe but due to timing/training it was not performed as requested. This resulted in unusual water parameter reading on the trial run. The cause of the abnormal readings was due to the failure of staff removing the caps from the probes. Other issues were discovered on the trial run of shark bag loading. The design of the transport opening did not take into the account the need bezel/nib for supporting the pressurized lid. The opening requested was 4.1 m x 1.5 m for bag be lowered unencumbered. A 100mm bezel/nib supporting the lid required the crossmembers of the bag required adjustment for lowering into the transport. Other minor issues include a few leaks caused by staff using unprotected plumbing for steps or handles and a short stand pipe for probes. Silicone was used for repairing the leaks and a taller stand pipe was installed.

The achievements far exceeded the minor issues. 17 Interstate Transports, a minimum of 1000 km each was conducted with little issues. Travel time was maximized using technology to allow full time monitoring of animals, water quality and life support. The use of the shark bag enabled the loading and unloading of large mature Grey Nurse Shark without being removed from the water. The size of the transport allowed for natural swimming behavior of the sharks for the entire transport. Blood chemistries were better on long transports than shorter transport meaning the animals showed reduced stress over the transport. The new systems where the sharks were relocated immediately showed drastic improvements resulting in amazing exhibits and guest satisfaction. However, the most important achievement was the moving thousands of animals thousands of kilometers that included six GNS ranging from 200-250kg, 3-3.5m long, 30-40 years old with zero mortalities.



NITRIFICATION EFFICACY WITH SUPPLEMENTAL PHOSPHORUS AND ORGANIC CARBON

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Introduction

Chemical cycling of newly-constructed life support systems (LSS) through the use of ammonium salts or urea is standard practice in the aquarium industry. These artificial sources of nitrogen have long been used to facilitate growth of ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) in biofilters prior to the introduction of teleost fishes and other aquatic life, though the process has its limitations. All too often the cycling period essential for good water quality and animal health is cut short or otherwise constrained by the rigors of construction schedules, forcing aquarists and LSS operators to expedite these microbiological processes as much as possible. Cycling of nitrite typically is typically more problematic than ammonia as NOB communities develop more slowly than AOB and must compete for space in biofilms. Though the cycling process promotes chemoautotrophic nitrification most species of NOB are facultative organoautotrophs, and as such can utilize organic compounds as the electron donor in nitrification (Prosser, 1990, Uemoto *et al*., 2014).

During the renovation of the historic Dallas Aquarium at Fair Park in 2009-2010, aquarists were placed in a similar predicament as the rigors of construction led to the time reserved for cycling and stocking of exhibits being constrained to a miniscule period. Two of the largest exhibits, a 220,000-liter shark tank and a 34,000-liter stingray touch tank were among the first to begin cycling with multiple doses of bacterial inoculum from multiple manufacturers, but even after 40+ days the biofilters of both remained unable to effectively process nitrite (measured as NO_2^-) to levels safe for teleost fishes. During this time, additional cultures of NOB were added, temperature was elevated, and diligent additions of inorganic carbon were made, though the biofilters were stalled and unable to complete cycling in the constrained timeframe. During this period the authors received communications from colleagues and microbiologists that each suggested potential deficiencies or problems with the biofilter.

Two of these suggestions stood out as more plausible and rooted in empiricism, namely that additions of organic carbon and phosphorous may alleviate sluggish nitrification by providing a greater diversity of carbon or compensate for an element limiting the process, respectively. One microbiologist suggested that a sugar dose of “about a handful” per thousand gallons might be sufficient to jump-start the process (Botto, pers. comm.). In another case an industry colleague described a condition termed “phosphate block”, where phosphorous is lacking and a suggested addition of a drop or two of phosphoric acid per gallon to correct the phosphorus deficiency and resume nitrification (Sweet, pers. comm.).

The role of phosphorous in nitrification was first described by Purchase (1974) from phosphorous-poor Rhodesian soils. Purchase not only described the inhibition of nitrification, but remarked that the effect was especially pronounced on the nitrite-oxidizing bacterial communities (1974). This construct was first put into practical application in aquatic nitrification in 1998, when

Kors *et al.* described a scheme for supplementing phosphate into wastewater to increase ammonium removal capacity and reduce incidences of nitrogen breakthrough in their product water. When this wastewater treatment process was treated with phosphorous, a rapid increase in nitrogen removal capacity was seen (approximately 50%) and ammonium dropped to near-zero (Kors *et al.*, 1998). Numerous other studies of wastewater applications have also found a significant relationship between phosphorous and nitrification (Lee *et al.*, 2001, Patureau *et al.*, 2001).

Attempting to quantify these two suggested protocols into a standard dosage some *approximate* quantifications can be constructed. A handful of sugar (sucrose) is roughly 75 g, giving a target concentration of approximately 20 mg/l of sugar, an effective carbon dose of 8.5 mg/l. Assuming one drop is 0.05 ml one can calculate that 1-2 drops glacial phosphoric acid is equal to a dose of 0.55-1.1 mg/l phosphoric acid. Following this, it is logical to conclude that a sugar dose of 75g/1000 gal and/or 2.1-4.3 mg phosphoric acid/1000 gal could be useful in chemical cycling of exhibits. Sucrose and other sugars are readily available, but glacial phosphoric acid is less obtainable on short notice, to this end a commercially available substrate was identified that was food-grade, inexpensive, safe for personnel to handle without PPE, and contained a ratio of carbon to phosphorous (C:P) close to the intended dosing schedule. The selected commercially available substrate contained roughly 108 mg/ml carbohydrates (approximately 9-13 mgC/ml) and an amount of phosphorous equivalent to 396 μg/ml phosphate. The chosen adjunct is commonly sold under the trade name Coca-Cola™(Coca-Cola Company, Atlanta, GA); the author also finds it a preferable adjunct to bourbon and/or rum when the quality of said spirit renders it unable to be consumed neat.

Methods

Water Quality Analysis

All analyses were performed on a Hach DR2800 spectrophotometer: ammonia was measured as NH_3-N and recorded as NH_3 via a Hach DR2800 spectrophotometer using the Salicylate method (Hach no. 10205), nitrite was measured as NO_2-N and recorded as NO_2^- using the diazotization method (Hach no. 8507), Nitrate was measured using the dimethylphenol method as NO_3-N and recorded as NO_3^- (Hach no. 10206). All glassware used in reagent reactions and cuvettes used in analysis were washed with 6M HCl prior to use, triple rinsed with DI water, and then triple rinsed with sample water. When the concentration of the ion being measured exceeded the method detection limit (MDL) of the reagent used, a 50% dilution was made with DI water and the diluted sample analyzed.

Small-Scale Validation Trials

Twelve (12) 20l (5 gal) food-grade HDPE plastic containers and biomedia (1" Polyethylene Bio-Balls™, Aquatic Eco Systems) were sterilized with 500 mg/l Cl^- (sodium hypochlorite) and neutralized with sodium thiosulfate. Each container was filled with 18 l freshly mixed artificial sea water (Instant Ocean™) at 30 ppt and given 100 cm^2 of biomedia (50% positively buoyant, 50% negatively buoyant), that had been inoculated for 48 h with a commercial nitrifying bacteria culture (Micro-Tes AWT-1™).

Additions of 20mg/l sucrose, 3.96 mg/l H_2PO_4, and 1ml/l Coca-Cola™ were each added to three 20 l aquaria during the validation. Four stock solutions were prepared in such a fashion

as to be colorless dilutions: a 0.5mg/ml sucrose solution, a 19.7 μg/ml phosphoric acid solution, a 5% aqueous solution of Coca-Cola™, and a control that was RO/DI water. The proportions of sucrose and phosphoric acid in the stock solutions was carefully chosen to match the sugar and phosphoric acid concentrations of the Coca-Cola™ solution. The stock solutions were labeled A, B, C, & D by a volunteer and the identity of each sealed in an envelope so that neither the author or the aquarists performing the water quality analyses would know which tank had been dosed with which additive until after all testing had been complete.

The containers were all placed in a recirculating water bath and a heater to ensure they were kept at a consistent temperature of 33ºC, salinity was adjusted back to 30 ppt twice weekly with DI water. Aeration was not provided to ensure that oxygen exposure to the water was not a variable, and the replicates were not buffered after day 1 to increase pH and alkalinity for the same reason. Each tank was given an initial dose 64 mg/l NH_4Cl (equivalent to 1.0mg/l NH_3) on day 1, and a second dose on day 25. 100 mg of a 1:6 sodium carbonate/sodium bicarbonate buffer was added on day 1 prior to ammonia addition. Stock solutions A, B, C, & D were dosed (400 ml each replicate) on days 1 and 25 of the trial; samples were taken weekly for analysis of ammonia, nitrite, and nitrate as described above.

Large-Scale Trial

A 220,000-liter shark tank had been cycling for approximately 40 days; the system contained mechanical filtration, biological filtration, ultraviolet sterilization, and foam fractionation, and had an approximate turnover rate of 45 minutes. An initial dose of NH_4Cl was added to raise the NH_3 concentration to 1.0 mg/l, and additional equal doses were added weekly thereafter whenever NH3 was measured below 0.10 mg/l. An initial inoculation of nitrifying bacteria was made (50 gallons of Micro-Tes AWT-1™) on day one and after 21 days of less than optimal performance a second commercial bacterial culture was added (15 gallons of Fritz Industries Turbo-Start 900™). On day 35 of cycling an addition of 30 gallons nitrite-only bacterial culture was added (Micro-Tes). On day 46 of cycling 58 l (a dosage of 1 ml/gal) of a readily available food-grade sugar/phosphoric acid solution (Coca-Cola™) was added in an attempt to revive growth of NOB in the seemingly "stalled" biofilter. Additional, smaller additions of this adjunct were made following the initial drop in nitrite as NO_2^- levels rose above 0.200 mg/l, and additions were discontinued once live animals were added.

Data Analysis

Results of weekly water quality analyses were averaged to provide a mean; these data were multiplied by the number of days between analyses to calculate cumulative measures of nitrite in each replicate. The resulting measure expressed cumulative biofilter NOB oxidative capacity (or lack thereof) as nitrite milligram-days (mg-d NO_2^-), which approximate the area under the curve (AUC) of nitrite plotted graphically against time. A series of 2-sample t-tests (α=0.05) were performed to assess the significance of the results using the =TTEST function of Microsoft Excel. Results were plotted graphically using Veusz 3.0.1 (https://veusz.github.io).

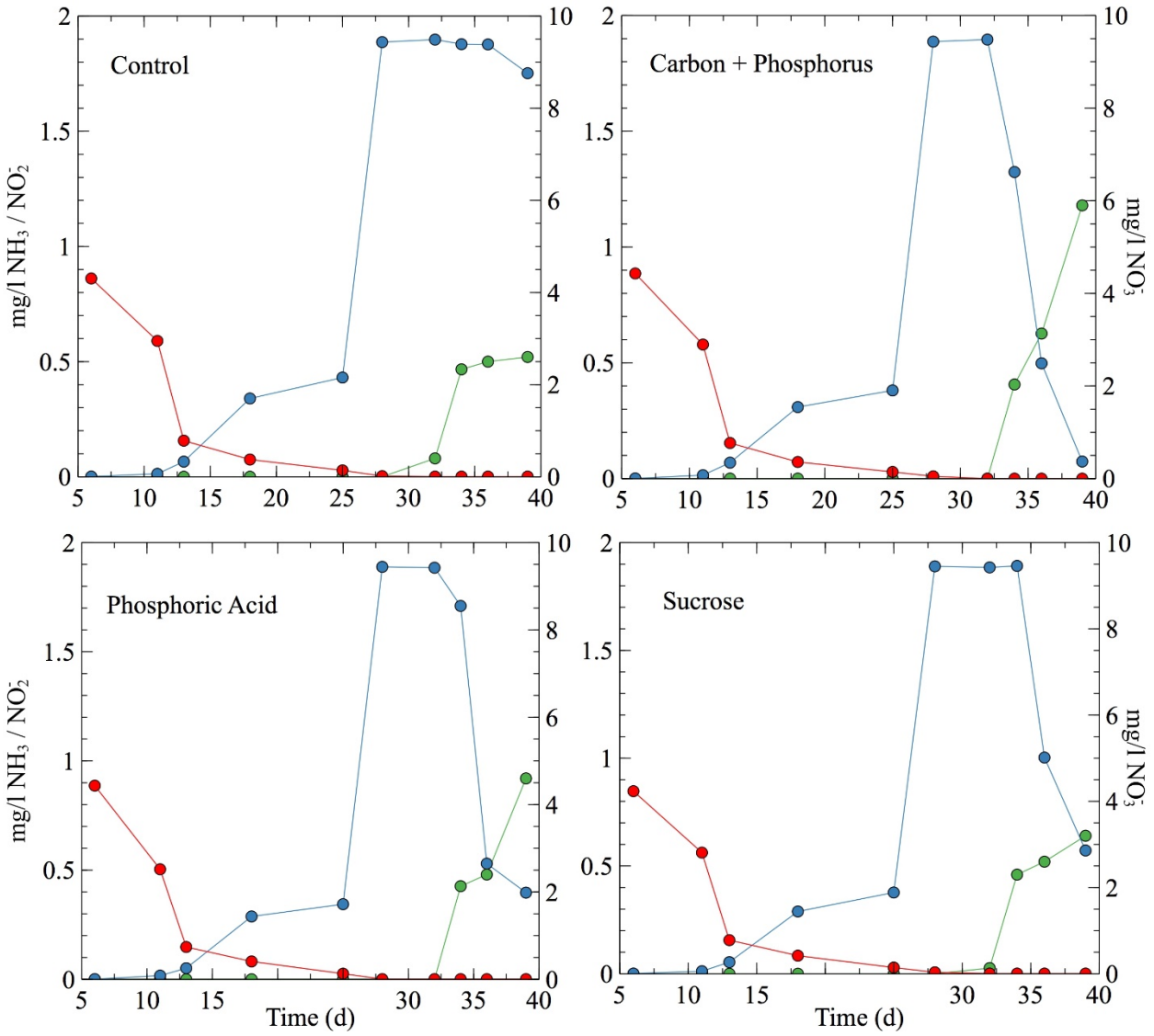

Figure 1. Mean ammonia (green), nitrite (blue), and nitrate (green) levels among n=12 replicates containing 20l artificial sea water and 100cm^2 of biomedia.

Results

While there was some variability in the small-scale trials, when averaged the process produced relatively normal levels of nitrogenous species, as would be expected (Table 1). Ammonia dropped to near zero in the first 3 weeks, nitrate had a corresponding inverse rise to ammonia, and nitrite curves were approximately parabolic (See Figure 1). When the nitrite curves are plotted together on one graph, a distinct difference in the residence time of nitrite can be observed in the control as compared to the systems dosed with sucrose, phosphoric acid, or Coca-Cola™ (Figure 2).

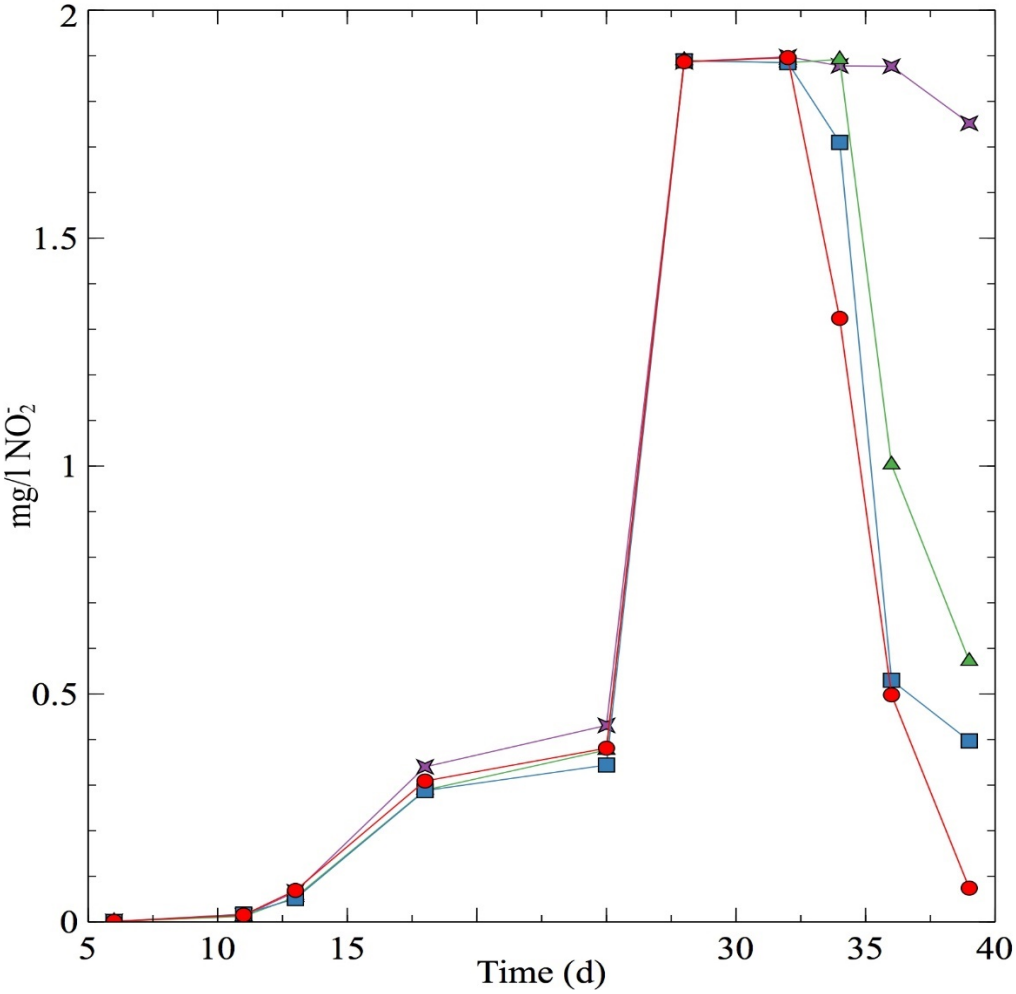

Figure 2. Mean nitrite (NO_2^-) curves for n=12 replicates containing 20l artificial sea water and 100cm^2 biomedia. Purple (cross marker) indicates the control group, green (triangle marker) indicates the sucrose group, blue (square marker) indicates the phosphoric acid group, and red (round marker) indicates the carbon+phosphoric acid substrate (Coca-Cola™) group.

When cumulative nitrite was calculated, the control group had a total of 40.25 mg-d NO_2^-, the sucrose group had a total of 32.96 mg-d NO_2^-, the phosphoric acid group had a total of 30.28 mg-d NO_2^-, and the carbon-phosphoric acid substrate (Coca-Cola™) had a total of 28.30 mg-d NO_2^-. These data represent a deviation from the control group of roughly 30% for the carbon-phosphoric acid substrate, 25% for phosphoric acid, and 18% for sucrose (Table 1 & Figure 3).

The differences in AUC mg-d NO_2^- between these replicates in chemical cycling of nitrogen are subtle, but distinct, as plotted in Figure 4 together, and in Figure 5 with AUC shaded to illustrate the differences. A 2-Sample t-Test revealed that the cola and phosphoric acid treatments differed significantly from control (α=0.04, both), but despite seeing reductions of 18-25% in mg-d NO_2^-, the sucrose (α=0.09) and phosphoric acid (α=0.26) groups did not differ significantly from the cola addition. Phosphoric acid was significantly different than control (α=0.09), but sucrose was not (α=0.07). A matrix of these relationships is displayed in Table 2.

Table 1. Mean Ammonia, Nitrite, and Nitrate Levels of Replicates (n=12) During Small-Scale Trial; and Cumulative Nitrite-Days of Each Variable. (Ck=Coca-Cola™, Pa=Phosphoric Acid, Su=Sucrose, Ct=Control).

Day	Coca-Cola™			Phosphoric Acid			Sucrose			Control			Cumulative NO_2^- Days			
	NH_3	NO_2^-	NO_3^-	NH_3	NO_2^-	NO_3^-	NH_3	NO_2^-	NO_3^-	NH_3	NO_2^-	NO_3^-	Ck	Pa	Su	Ct
3	0.89	0.000	0.0	0.89	0.000	0.0	0.85	0.000	0.0	0.86	0.000	0.0	0.00	0.00	0.00	0.00
6	0.58	0.015	0.0	0.50	0.017	0.0	0.56	0.012	0.0	0.59	0.013	0.0	0.05	0.05	0.04	0.04
11	0.15	0.069	0.0	0.15	0.051	0.0	0.16	0.054	0.0	0.16	0.066	0.0	0.35	0.26	0.27	0.33
13	0.07	0.309	0.0	0.08	0.288	0.0	0.08	0.289	0.0	0.08	0.340	0.0	0.62	0.58	0.58	0.68
18	0.03	0.381	0.0	0.03	0.344	0.0	0.03	0.377	0.0	0.03	0.431	0.0	1.91	1.72	1.89	2.16
25	0.00	1.887	0.0	0.00	1.889	0.0	0.00	1.890	0.0	0.00	1.887	0.0	13.21	13.22	13.23	13.21
28	0.00	1.896	0.0	0.00	1.885	0.0	0.00	1.885	0.0	0.00	1.898	0.0	5.69	5.66	5.66	5.70
32	0.00	1.324	2.0	0.00	1.710	2.1	0.00	1.892	2.3	0.00	1.878	2.3	5.29	6.84	7.57	7.51
34	0.00	0.498	3.1	0.00	0.530	2.4	0.00	1.003	2.6	0.00	1.877	2.5	1.00	1.06	2.01	3.75
36	0.00	0.074	5.9	0.00	0.397	4.6	0.00	0.572	3.2	0.00	1.752	2.6	0.15	0.79	1.14	3.50
39	0.00	0.017	7.1	0.00	0.035	5.9	0.00	0.196	4.8	0.00	1.124	2.9	0.05	0.10	0.59	3.37
												Total:	28.30	30.28	32.96	40.25
												Percent of Control:	70%	75%	82%	-
												Percent Reduction:	30%	25%	18%	-

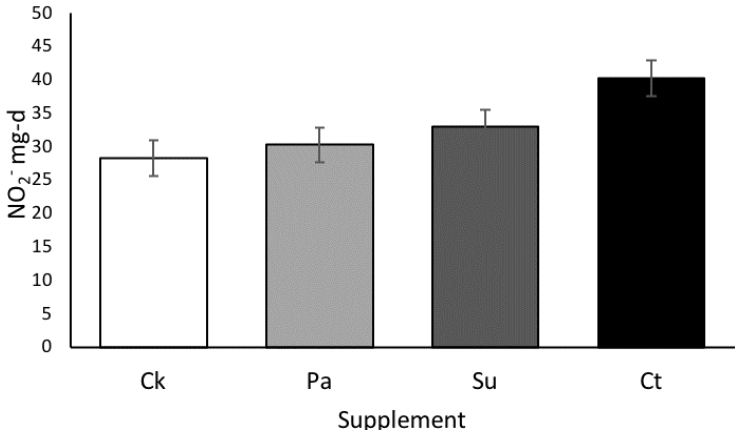

Figure 3. Mean total milligram-days of nitrite (mg-d NO_2^-) for n=12 small aquaria supplemented with a carbon-phosphoric acid (Coca-Cola™) formulation (Ck), phosphoric acid (Pa), Sucrose (Su), and a control (Ct) supplemented with DI water. Error bars denote standard error.

Table 2. 2-Sample t-Test matrix for small scale trials.

	Ct	Su	Pa	Ck
Ck	0.04	0.09	0.26	-
Pa	0.04	0.04	-	0.26
Su	0.07	-	0.04	0.07
Ct	-	0.07	0.04	0.04

a < 0.05 a > 0.05

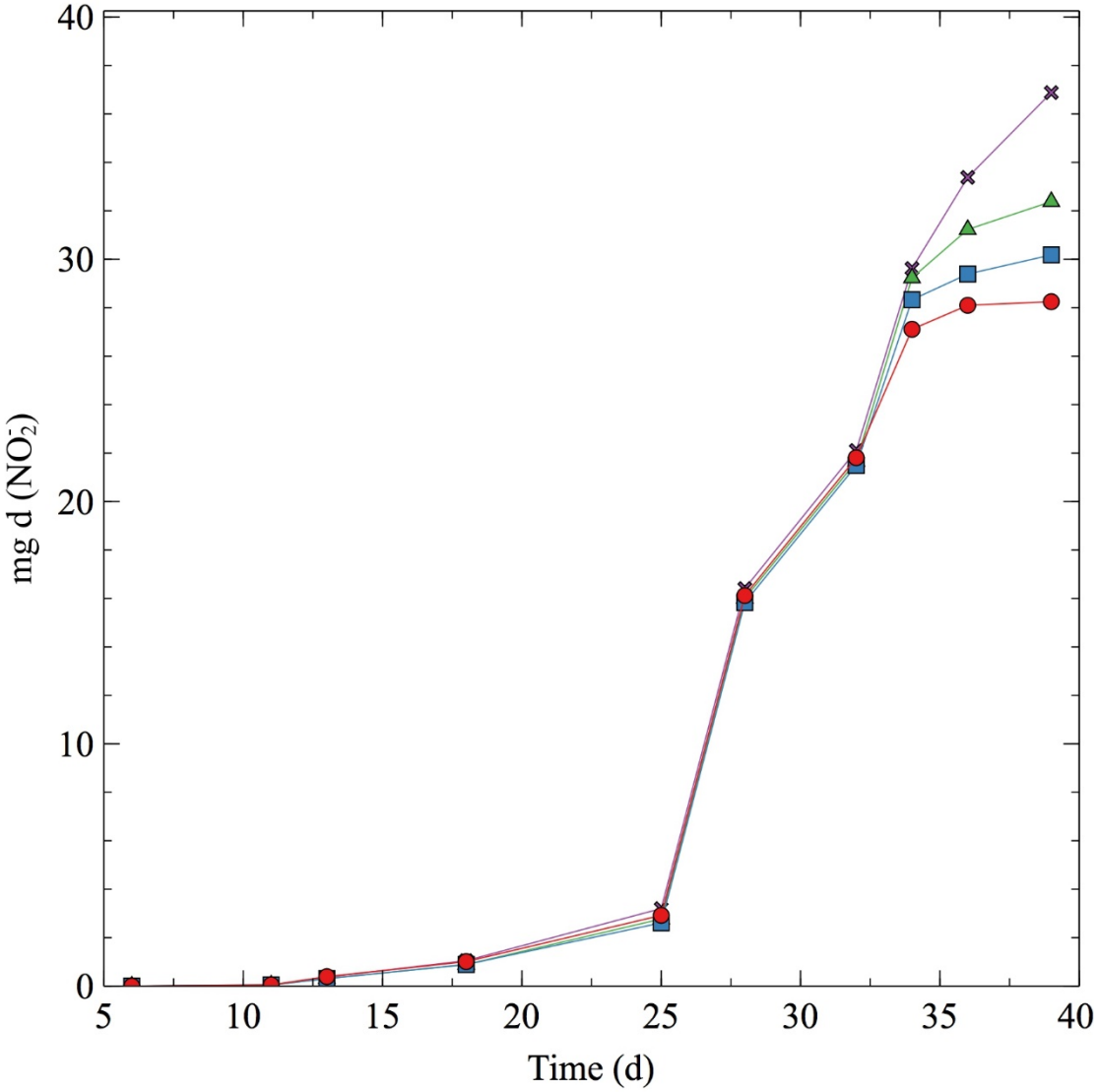

Figure 4. Cumulative milligram-days of nitrite (mg-d NO_2^-) for n=12 replicates containing 20 l artificial sea water and 100 cm^2 biomedia. Purple (cross marker) indicates the control group, green (triangle marker) indicates the sucrose group, blue (square marker) indicates the phosphoric acid group, and red (round marker) indicates the carbon+phosphoric acid substrate (Coca-Cola™) group.

In the large-scale trial, the organic carbon-phosphoric acid solution (Coca-Cola™) was added after 46 days of chemical cycling with traditional methods. A precipitous decline was seen immediately (Figure 6). The nitrite levels, which had been above the range of detection (2x MDL), even for a sample diluted 50% with DI water, fell by approximately 0.20mg/l NO_2^- per 24-hour period for the first 48 h, followed by a steady decline to under 0.10 mg/l NO_2^- (the level considered to be indicative of a well-functioning biofilter and balanced LSS). This initial rapid decline represents a nitrification capacity of 44 kg/d NO_2^- either being oxidized to nitrate or otherwise utilized by the microflora of the tank.

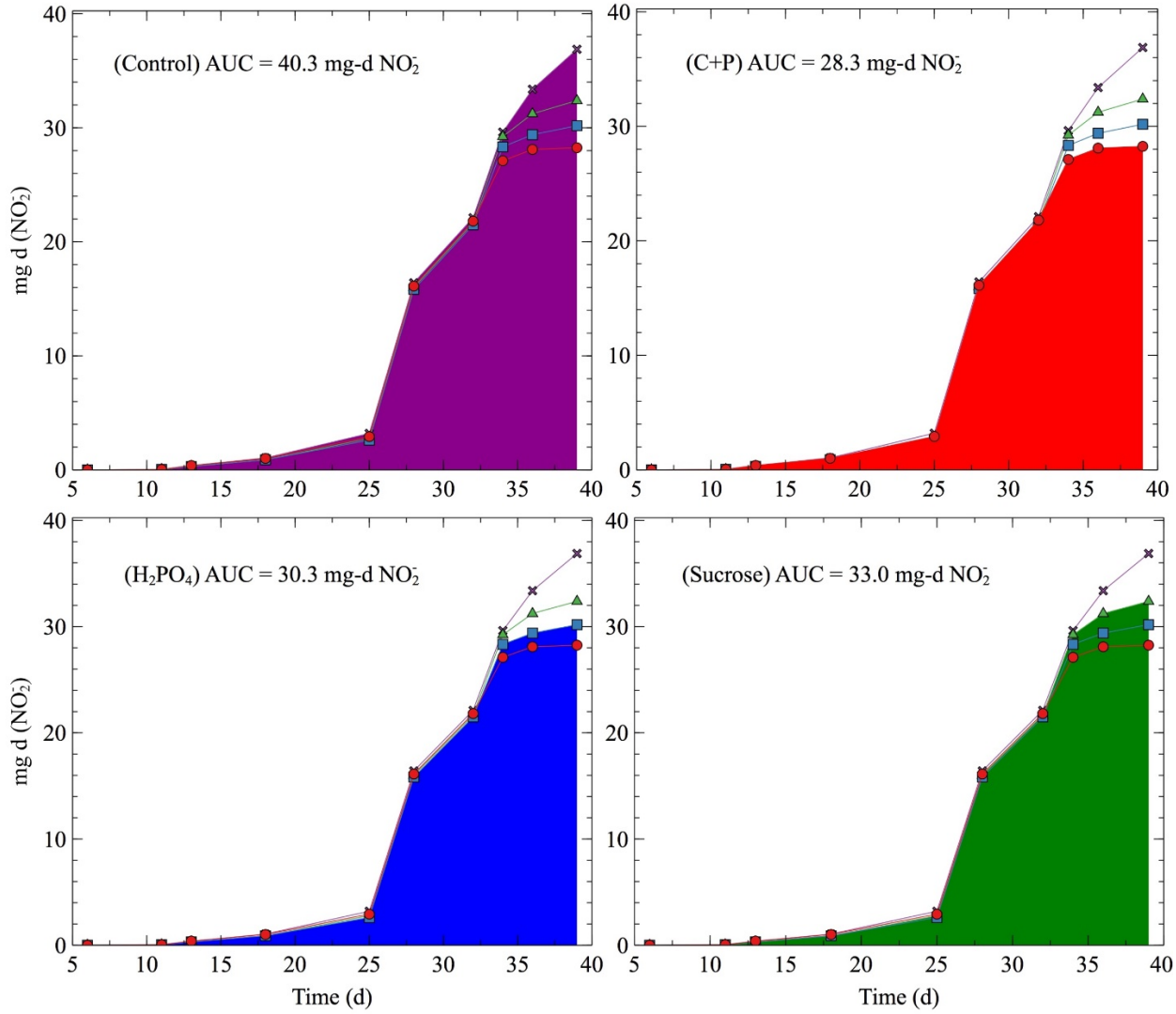

Figure 5. Area under the curve (AUC) plots representing cumulative milligram-days of nitrite (mg-d NO_2^-) for n=12 replicates containing 20 l artificial sea water and 100 cm^2 biomedia. Purple (cross marker) indicates the control group, green (triangle marker) indicates the sucrose group, blue (square marker) indicates the phosphoric acid group, and red (round marker) indicates the carbon+phosphoric acid substrate (Coca-Cola™) group.

Discussion

The science of keeping captive fishes and invertebrates has evolved rapidly over the last half-century, but in many respects our methods are still carried out dogmatically without complete understanding of the processes involved. For far too many years it was assumed (and some texts still regurgitate) that ammonia is oxidized to nitrite in closed systems by *Nitrosomonas spp.* and nitrite is oxidized to nitrate by *Nitrobacter spp.*, yet we now know that entire microbial ecosystems are at play in the functioning of the biofilters that are so critical to husbandry. In a series of seminal papers 20 years ago, Hovanec began to establish that nitrification is much more complex than we previously thought, and that models assuming *Nitrosomonas* as the major nitrifying spp. need to be revised (Hovanec and DeLong, 1996). In 1998 Hovanec defined the major role of *Nitrospira* spp. in nitrite oxidation in closed systems, and further established that the chemolithotrophic NOB

are a diverse group of microbes. And many species of nitrifying archaea were shown to play a role in ammonia oxidation (Francis *et al.*, 2005, Mincer *et al.*, 2007, Hayatsu, 2010), broadening the microbial phylogenetic diversity by bringing an entire new kingdom into the fold. These nitrifying archaea have also been identified from aquaria (Sauder *et al.*, 2011). In 2007 Tal *et al.* found that bacteria in a biofilm from an aquaculture system were actively oxidizing ammonia and nitrite to nitrate simultaneously through the Annamox reaction, representing an exciting novel metabolic pathway for nitrification in closed systems. Recent investigations of the microbiome of aquaria have revealed they have surprisingly complex microbiomes that show dramatic fluctuations in microbial composition over time (Patin *et al.*, 2018).

In this investigation, the cola additions were certainly found to be effective in "jump-starting" a sluggish biofilter, though the data collected from the small-scale trials is more suggestive as to the mechanism behind the practical results. Addition of a carbon/phosphoric acid solution had a significant difference from control, but it was not significantly different from the phosphoric acid or sucrose groups, indicating that there is not a synergistic effect in adding both adjuncts to a system during cycling. While not statistically rigorous, there may be some benefit to adding both phosphoric acid and organic carbon in tandem in a practical setting, as mg-d NO_2^- were reduced by 5% over phosphoric acid alone and 12% over sugar alone, and every day counts when new exhibits are scheduled to open.

It is possible that the effect of sugar or phosphoric acid in these trials could be due to blooms in waterborne bacteria and/or algae exploiting a newfound source of carbon or phosphorous. The Redfield Ratio (Redfield, 1958) is an ecological construct describing nutrient flow in the world's oceans that is often applied to microbial growth in closed systems in practical ways such as nitrogen export in DOC-limited systems through ethanol supplementation (Delbeek and Sprung, 2005). While recent studies have refined Redfield's equations (Arrigo, 2005), for our purposes the original ratio of 106:16:1 C:N:P is close enough to estimate the impact of microbial flora in nitrogen export. In the case of this particular shark tank, 220,000 l of volume with a nitrite concentration of 1.80 mg/l+, ammonia of 0.13 (TAN=2.45mg/l), and nitrate level of 16 mg/l there is a concentration of 20.25 mg/l of total nitrogen in the water column. Following the Redfield stoichiometry, we would need a dose of 134mg/l of organic carbon to completely remove the nitrogen from the system, far above the roughly 20mg/l DOC that resulted from the cola addition. If phosphorous were the limiting element, a phosphorous concentration of 1.27 mg/l would be required, again far higher than the 0.10 μg/ml dose contributed by the cola addition. Thus, we can assume that the mechanisms behind the efficacy of this method are not solely due to nitrogen export though a bacterial bloom conforming to Redfield stoichiometry, and must be the result of more complex metabolic pathways being activated either within AOB/NOB biofilms, or perhaps ecological interactions between microbe species.

Maximizing Cycling Efficiency

There are numerous factors that are known to either inhibit, or promote nitrification in closed systems. Elevated levels of ammonia are known to inhibit NOB but not AOB (Gieske, 2003), ergo overzealous feeding of AOB at the beginning of cycling may stall nitrification. As demonstrated above phosphorous is a limiting factor and promotes complete nitrification, and inorganic carbon sources may also speed cycling. There is evidence that both AOB and NOB are

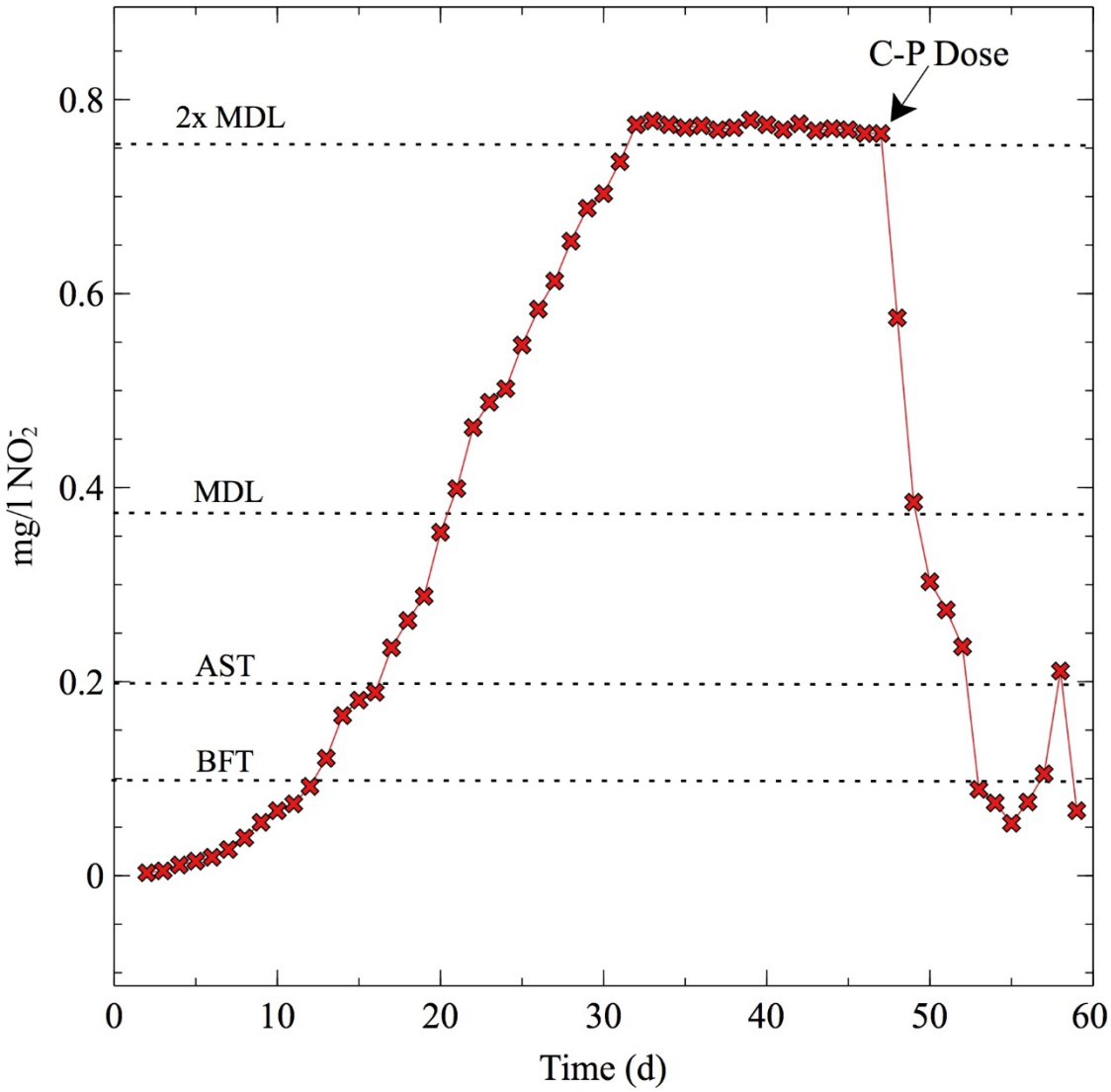

Figure 6. Results of a large-scale trial of a carbon and phosphoric acid substrate (Coca-Cola™) dosed at 1.0 ml/gal to a 220,000-liter shark tank in an attempt to alleviate stalled nitrification. The curve represents nitrite (NO_2^-) levels over time. The substrate addition is indicated by an arrow after 46 days of chemical cycling without appreciable nitrite oxidation. Vertical dotted lines indicate the method detection limit (MDL), double the limit (2xMDL), the animal safety threshold (AST), and biofilter functionality threshold (BFT).

inhibited by light, specifically light in the near-UV and blue spectra (300-375 nm, 400-475 nm) so avoidance of photoinhibition by shielding biofilters may promote establishment of nitrifying biofilms (Guerrero and Jones, 1996). Aside from oxygen, the most important chemical component in nitrification are bicarbonate ions, multiple studies have demonstrated the critical need for alkalinity maintenance during this process (Wet and Rauch, 2003). Salinity and temperature also play a factor; changes in salinity have shown effects on microbial diversity in biofilters (Grommen *et al.*, 2005), and warmer temperatures are not only conducive to increased rate of reaction, but also promote higher microbial diversity (Urakawa *et al.*, 2008).

Nitrite oxidation is often a slower process than ammonia oxidation in newly established biofilters, yet is critically important for animal health and welfare. Although the effects are less pronounced in seawater versus freshwater (Kroupova *et al.*, 2005), nitrite toxicity still has negative effects on marine fishes at sublethal concentrations (Medeiros *et al.*, 2015). In a modern aquarium or zoo where animal welfare is a paramount concern, stocking a tank with animals before a biofilter has demonstrated efficacy at oxidizing both ammonia and nitrite to safe levels should be considered unethical and unthinkable, so every possible strategy for maximizing chemical cycling should be employed.

Acknowledgements

Thanks are due to tankmen Lyssa Torres (SeaLife Arizona) and John W. Foster IV (Point Defiance Zoo and Aquarium), as well as Dr. Richard Cohen and Flora Cohen (Dallas Aquarium at Fair Park) for assistance in countless water quality analyses during this project. Thanks to Brian Nelson (National Aquarium in Baltimore), and Jay Hemdal (Toledo Zoo) for reports on the efficacy of this method at their respective facilities. Thanks to Stephan Walker (Dallas Aquarium at Fair Park, Ret.) for allowing his staff to pursue off-beat (i.e. insane) experiments. Thanks to Dr. Dave Hudson (Maritime Aquarium) for review of the manuscript. Special thanks are due to Doug Sweet (London State Fish Hatchery) and Dr. Bill Botto (Micro-Tes) for collectively providing the initial inspiration for this project. And of course, thanks are due to Dr. John Stith Pemberton for devising the commercially available sucrose-phosphoric acid substrate used in this experiment in 1886.

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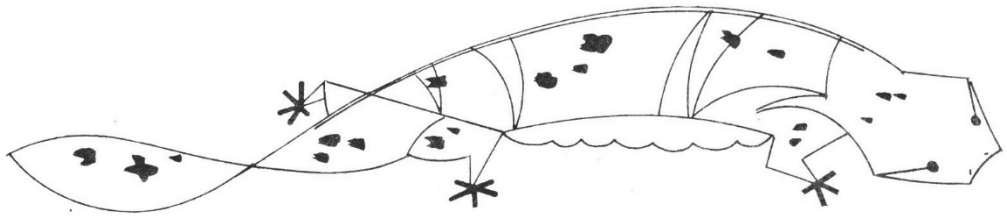

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Monday, 13 May 2019
8:00 – 5:00 Presentations and **Aquatic Invertebrate TAG Reporting Meeting**

Tuesday, 14 May 2019
8:00-5:00 Presentations and **Marine Fish TAG Reporting Meeting**
Icebreaker at the Hyatt Regency

Wednesday, 15 May 2019
8:00-12:00 Presentations
1:00-9:00 Zoo Day and Aquarist Olympics

Thursday, 16 May 2019
8:00 – 5:00 Presentations and **Fresh Water Fish TAG Reporting Meeting**
PINS Mechanical Networking Event

Friday, 17 May 2019
8:00-1:00 Presentations

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EFFECT OF SCREEN SIZE ON *Leuckartiara spp.* MEDUSA GROWTH

Valerie Kleitman, Drifters Gallery Intern
vrk25@humboldt.edu

Monterey Bay Aquarium

Abstract:

Monterey Bay Aquarium is a non-profit public aquarium in Monterey, California, known for its focus on regional marine ecosystems. One unique species on display in the Drifters Gallery, is a hydroid medusa named *Leuckartiara* spp. This jelly has been cultured and placed on display here, since it was first collected locally in 2016, but little was known about how to optimize their growth rate and a limited number of individuals were able to be produced at the aquarium with the current culturing methods. In order to study the effect that different mesh-screen sizes, used during construction of the aquaria, have on the survival and growth of the *Leuckartiara* medusa, three screen sizes were chosen to be tested: 400 microns(μm), 125 μm, and 53 μm. This was based on the previously recorded sizes of the cultured prey items being used, *Artemia franciscana* nauplii and *Brachionus plicatilis,* and includes the most routinely used size, 400 μm. Although the results of this comparative growth study did not show any significance statistically, the average growth rate, graphically, was notably higher when the *Leuckartiara* medusa were placed in an aquarium with a 53 μm mesh-screen for the duration of the study. The success of this study has not only allowed for aquarists here to populate the display tank with more, healthy individuals, the same aquaria, used for this study, are still being utilized to perform grow-outs of this species at Monterey Bay Aquarium.

Introduction:

A leader in ocean education and conservation, Monterey Bay Aquarium is considered one of the top public aquariums in the world. Although there are many exotic species on display, the primary focus is to exhibit regional species and to inspire conservation of the ocean. With captive breeding programs in place for over 50 different species, it is now possible to populate exhibits at the aquarium without the need to collect from the wild. In addition to developing husbandry methods for jellies that are now used by aquariums around the world, biologists here have been the first to describe the life cycles of 5 different jelly species, which can commonly be seen on display in the Monterey Bay Aquarium Drifters Gallery.

One species you may see in the Tiny Drifters section of the "jelly hallway", is a small, rocket ship-looking jelly, with electric orange highlights. This unique Cnidarian, a *Leuckartiara* spp., is the medusa stage of a colonial athecate hydroid, which can be found local to Monterey Bay, California and is also distributed throughout many parts of the world. These unique invertebrates thrive in a three-stage life cycle, which includes a planula, polyp, and medusa stage. Medusa are typically seasonal organisms, but when cultured in a lab setting, the sessile hydroid colony can be triggered to produce medusa with simple changes to diet.

The result has allowed for aquarists at Monterey Bay Aquarium to put these unique jellies on display, but due to the large requirements of studying jellies and the important work done by the prestigious aquarists, there is often minimal time allotted for new research and

experimentation. Luckily, through the Monterey Bay Aquarium Internships program and the Jellies Propagation & Research program, aspiring researchers and ocean conservationists (like myself) have the opportunity to work hands on in the Drifters Gallery, aside the senior aquarists, as well as having the opportunity to carry out and present a research project related to the relationship of cultured foods to jelly growth.

In many aquaculture techniques a 400 μm mesh-screen is placed in a way that covers the out-flow port of open-system aquaria, in order to protect organisms from exiting undesirably. It is now determined that the screen can be used to control other factors in the system, related to the growth of the organism being contained. This is due to the fact that altering the screen micron size can increase or decrease the clearance rate of many zooplankton, which are commonly used in aquaculture for feeding purposes. This is why it was thought that by changing the micron size of the screen being used, when building the aquaria, a higher level of survival and growth could be observed in the *Leuckartiara* medusa, during early development.

In order to study the effect of different screen sizes on the *Leuckartiara* medusa, three screen sizes were chosen to be tested (400 μm, 125 μm, 53 μm), based on measurements previously taken of the chosen prey items, *Artemia franciscana* nauplii (Brine shrimp) and *Brachionus plicatilis* (Rotifers) (Båmstedt et al, 2001). Three replicate aquaria were built for each of the three screen sizes, and one control tank was built using the standard 400 μm screen size. This made a total of 10 hand-crafted aquaria, which were installed semi-permanently into one of the Drifters Lab's wet tables.

Methodology

Prior to Study:

The *Leuckartiara* spp. cultured at Monterey Bay Aquarium was collected by Wyatt Patry in Monterey Bay, California in 2016. The medusa was spawned on the vessel and later placed in the Monterey Bay Aquarium Drifters Lab for the planula to settle and colonize. The species was currently being utilized by senior aquarist Michael Howard, who was rearing them for display in the Tiny Drifters section of the Drifters Gallery.

10 test aquaria were constructed by hand, carefully using silicone to adhere the different size screens to the inside of acrylic aquaria, each with a volume of 6.5 Liters. Three holes were also drilled on the edge of the aquaria, placed along a straight line, where the aquaria water-level is preferred. All 10 aquaria were then installed into the wet table using a previously built manifold. Rubber tubing was connected and fixed to each aquarium in such a way that the inflow was near the center of the aquaria. The end of each tube was also cut at a diagonal angle, versus straight across, in order to limit the surface turbidity and production of bubbles on the surface.

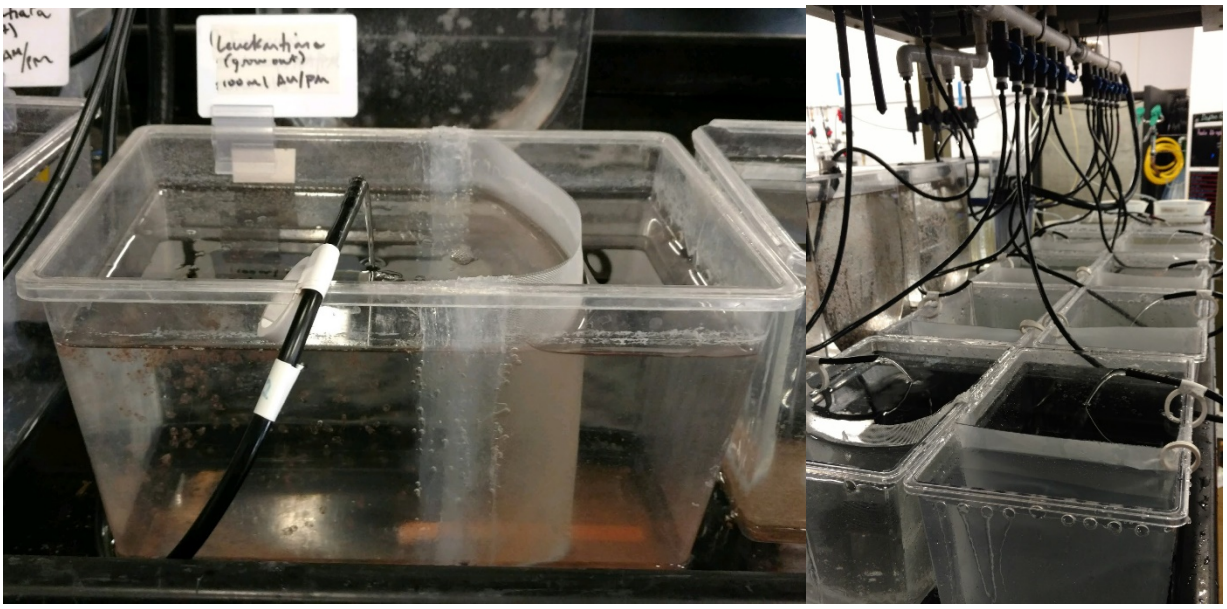

Figure 1. Aquaria setup showing mesh-screen placement inside, and he installation of all ten aquaria in the wet-table.

To produce medusae to be used in this study, the *Leuckartiara* hydroid colony was fed 100 mL of Rotifers in seawater and algae daily. Newly budded medusae were collected in an ephyrae catch aquaria, which was placed below the outflow of the *Leuckartiara* colony's aquaria. A pipette was then used to remove, count, and place 50 individuals from the ephyra catch, at random, into each of the 10 constructed aquaria previously assembled into the wet table. All 10 test aquaria were provided with a constant supply of fresh seawater, at 12° Celsius and a with a flow of 0.25 gpm, through a previously constructed manifold.

Feeding Protocol:

The 9 test aquaria were fed 25 mL of *Artemia* nauplii in a seawater solution and 50 mL of Rotifers in a seawater solution daily during the "PM" feeding time, which occurs between 1:00 PM-4:00 PM. The 1 control test aquaria was fed 50 mL of *Artemia* nauplii in seawater solution daily during both the "AM" feeding time, which occurs between 8:00 AM-10:00 AM, and the "PM" feeding time. 50 mL of Rotifers in seawater solution were also given to the control aquaria during at the "PM" feeding time. These cultured, live food species were harvested daily and distributed.

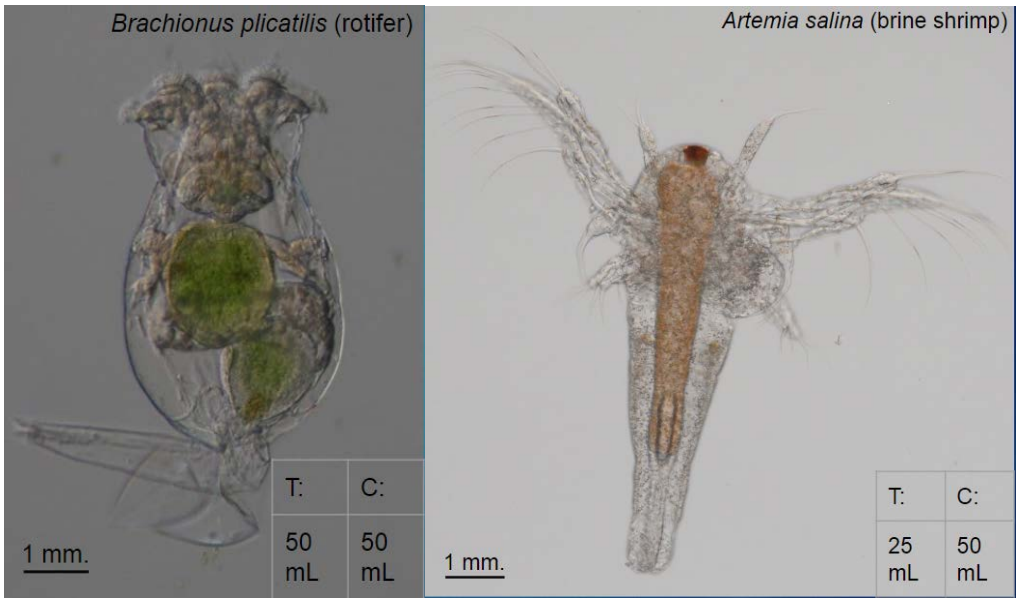

Figure 2. Types of live prey and volume fed per aquaria (T=test aquaria;C=control aquaria).

Sampling Protocol/Statistical Analysis:

10 individual medusae were collected at random from each of the 10 constructed aquaria. This was done using a modified pipette, and carefully transferring each individual to be photographed using a Zeiss microscope fitted with Canon camera. This was repeated weekly over a 7-week period. Calibrated measurements of bell height and bell width (from the outside edge of one radial canal to the outside edge of the opposite radial canal) were then taken using the computer program ImageJ. A two-way ANOVA test was then performed on the data using Microsoft Excel in order to compare growth of all three screen sizes tested.

Results:

The results of this comparative growth study showed that the average bell height was higher when the *Leuckartiara* medusa were placed in an aquarium with a 53 μm mesh-screen (Fig. 3). However, even though there was a trend seen graphically, this trend was not statistically significant, when tested using a two-factor ANOVA test (Bell height: $p = 0.081118$, Bell width: $p = 0.651582$). Survival rate of each aquaria showed a range of 6-50 individuals at the end of the study.

Discussion:

Although statistically speaking, there was no significant difference in bell height or bell width between screen sizes, the 53 μm screen did show a notable trend towards the fastest and most bell height growth, when compared to the 400 μm and 125 μm screen sizes (Fig. 3). This is thought to be because the 53 μm screen was the only size, out of the three tested, which retained both the *Artemia* nauplii and Rotifers with a 0% clearance rate. This meant that although these test aquaria were not being fed twice a day, like the control aquaria, they almost consistently had live prey items within the tank. In the case of the control aquaria, even though they were being fed twice during the day, based on the clearance rates previously determined, both species of live prey lasted only 60 minutes inside the aquaria and that became the only time frame available for feeding.

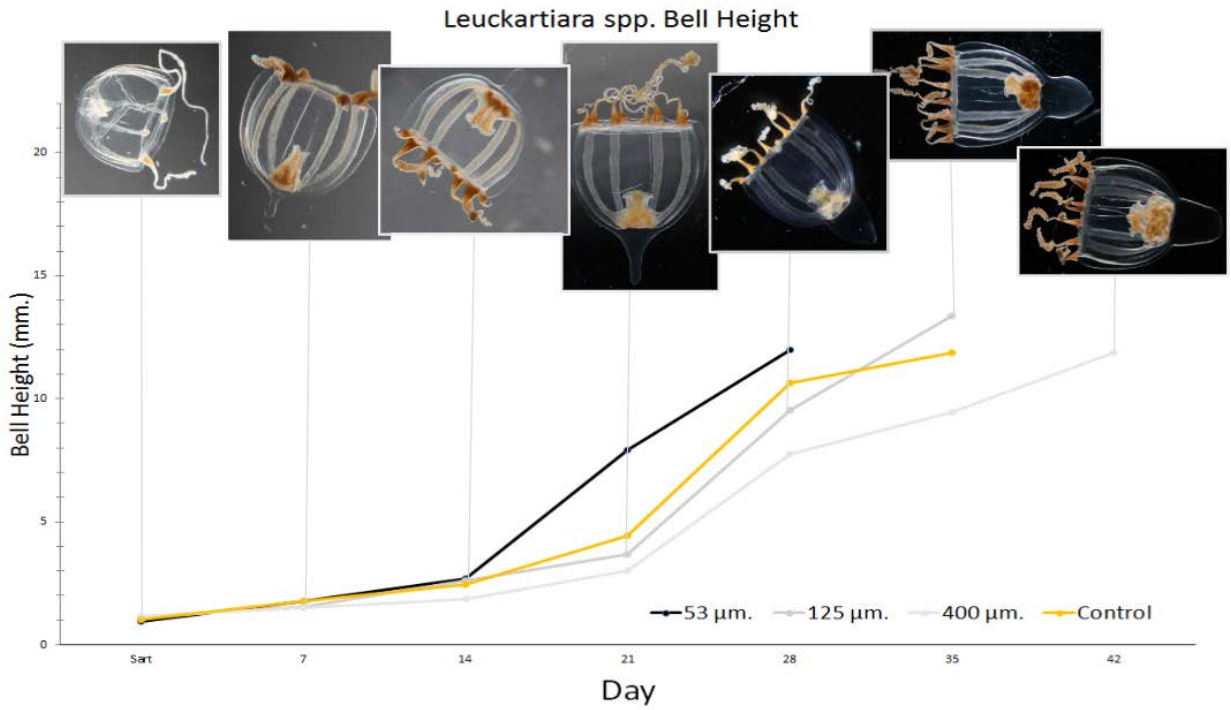

Figure 3. Average bell height, weekly of the *Leuckartiara* spp. Medusa over a 7-week time period. (Past week four, sizes of tanks, starting with 53 µm, were "graduated" and placed into a larger tank and removed from the growth study thus forth.)

Approximately the same clearance rate was observed in the aquaria fitted with 125 µm screen, although only Rotifers were tested due to the fact that Artemia nauplii should be larger than 125 µm. By principal, when using smaller screen sizes than the size of the live food, the live food will be retained within the system until they have been preyed upon or removed. This is why measurements of *Artemia* nauplii and Rotifers were taken prior to the study, along with their retention rates in the different screen sizes, so that appropriate sizes of screen could be chosen for this study.

Conclusion:

In the aquaculture industry, there is always something to clean or feed. So, the argument can be made for some cases, that choosing a mesh screen size to optimize the growth of the organism being reared is actually more valuable, in the long run, over any additional time being spent cleaning fouled screens. This is especially true if this cleaning procedure is already being implemented regularly in an aquaculture setting, and if the size of the aquaria is small enough that this can be done easily. Furthermore, live food, which is often very energy-costly to produce, can be utilized more efficiently with less flowing out of the system before it has a chance to be preyed upon.

With minor adjustments to common aquaculture techniques, huge benefits can be seen in the growth of developing hydromedusa. In this case, using smaller mesh screens decreased the time of growth to adulthood of the *Leuckartiara* medusae, while also increasing the integrity of the organism at adulthood. Furthermore, this allowed for enough healthy individuals to be raised in order to populate the exhibit to its full potential. Although there is still little physiological data

with which to study hydromedusa, their significance in planktonic communities is rising (Costello, 1987).

Acknowledgements:

The author would like to thank Monterey Bay Aquarium for providing the unique opportunity and environment possible to work on this study. Senior aquarists Thomas Knowles, Wyatt Patry, and Michael Howard were also essential to the success of this study, and it could not have been completed without their knowledge, expertise, and guidance. Special thanks would also like to be given to Zoe Kleitman, Karen Coral, the Drifters Gallery Volunteers/Interns, and the Monterey Bay Aquarium Assistant Aquarists for their dedicated work towards the continued success of this project.

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RAW 2018 ABSTRACTS
Regional Aquatics Workshop, May 14-18.
The Florida Aquarium, Tampa, FL.

AZA SAFE Shark Workshop - May 11-12th.
Florida Aquarium Coral School – May 12th.
AZA Aquatic TAG Steering Committee Meetings – May 13th.
Workshops and AZA Aquatic TAG Reporting Meetings - May 14th.

Tuesday, May 15th
Session 1: Coral Conservation & Partnerships 1
Moderator: Amy Tillman

Welcome and Introduction:
Roger Germann, The Florida Aquarium

Keynote Speaker:
Martin Moe

Challenges and Advancements in Cryopreservation of Staghorn Coral (*Acropora cervicornis*) Sperm.

C Adams MSc, [1] LM Penfold PhD,[1] S Graves, [2] K O'Neil MSc,[2] and M McKnight[2]

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Critically endangered staghorn coral (*Acropora cervicornis*) is threatened by storms, thermal bleaching, and disease in the Florida Keys. Ex situ propagation of coral for reseeding reefs could be enhanced by the use of cryopreserved sperm for best genetic combinations as well as preservation of valuable genetics for the future. Techniques for other coral species have been applied to *cervicornis* with success, with post-thaw sperm motility differences dependent on genotype, similar to other coral species. Eleven individual coral genotypes have been stored in a genome resource bank based at Florida Aquarium. Higher post-thaw motilities were achieved with a lower cryoprotectant concentration than is used for other coral species. A field-friendly freezing method proved successful in preliminary trials with 50% post-thaw motility. Future studies will investigate post-thaw fertility of sperm frozen using this technique. Development of a genome resource bank for *Acropora cervicornis* may aid in long-term genetic management of this species.

Sexual Reproduction, Settlement, and Growth of Threatened Caribbean Corals

Rachel Serafin, Keri O'Neil, Josh Patterson, and Kathryn Lohr

Seven Caribbean coral species are currently listed as Threatened under the U.S. Endangered Species Act. A major priority for recovery is mitigating threats to these corals such as disease, land-based pollution, ocean acidification, and increased ocean temperatures leading to more frequent bleaching. The past five years have seen rapid development of aquaculture as a complimentary tool to produce coral biomass and increase genetic diversity. Ocean-based coral nurseries now generate tremendous coral biomass for reef restoration through asexual propagation. However, there are low instances of successful sexual reproduction for Atlantic corals. To meet genetic diversity goals of important reef building corals, it is important to develop reliable techniques for sexual reproduction, juvenile settlement, and survivorship.

For the past several years the Florida Aquarium has been leading one such effort in partnership with the Coral Restoration Foundation and the University of Florida, among others. Coral larvae collected from the 2017 spawns of *Acropora cervicornis*, *A. palmata*, and *Orbicella faveolata* were settled ex-situ at the Center for Conservation in Apollo Beach, Florida. Six-week post-settlement survival rates for the three species range from 78-81% and are the highest achieved at this facility to date. This talk will provide details of current techniques and lessons learned over the years for ex-situ sexual propagation of these coral species.

Rescue, Husbandry, and Disease Treatment of Atlantic Pillar Coral *Dendrogyra cylindrus.*

Keri O'Neil, MS., Kathy Heym, DVM., Scott Graves

The Florida Aquarium participated in a multi-agency effort to survey and sample the remaining population of *Dendrogyra cylindrus* in the state of Florida. This coral species, along with many others, has suffered heavily from the recent outbreak of a disease known as white plague. A living genetic bank of this species was formed in several land-based aquarium facilities, including the Center for Conservation. During our time forming an archive of the species, various techniques of disease treatment and husbandry were tested on the species in order to stabilize the wild-collected fragments that often came from diseased locations. In coordination with the NOAA National Ocean Service, the Center for Conservation was able to clinically test one disease treatment that may be applied to wild populations in the future.

Sponsor: Aqua Logic

Session 2: Coral Conservation & Partnerships 2
Moderator: Mike Brittsan

Sponsor: Abyszz by Venotec

Developing Land-Based Coral Facilities to Stimulate Multiple Ex-Situ Broadcast Spawning Events Per Year for Reef Restoration.

Jamie Craggs[1, 2 & 3], Keri O'Neil[4], Scott Graves[4], Dr James Guest[5] & Dr Michael Sweet[1]

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In February 2017 the Horniman Museum & Gardens (HMG), London and the Center for Conservation (CFC), Florida entered a partnership to develop land based coral research facilities. Via the ex-situ production of sexually reproduced coral spat these facilities aim to support coral restoration initiatives in the Florida Keys. Using techniques developed at the HMG, where ex-situ gamete production has been induced in 17 species of *Acropora*, the CFC is constructing four climate-controlled systems that, from 2019, will facilitate multiple broadcast coral spawning event per year. Initially focusing on the US Endangered Species Act and critically endangered species *Acropora cervicornis*, the team at CFC will gain increased access to gametes per year and building on their current field knowledge, will explore opportunities to up-scale coral production for restorative purposes.

This presentation will cover some of the ex-situ broadcast spawning methodology developed at the HMG and construction progress at CFC.

Design and Durability of a Low-Maintenance Coral Nursery

Nancy Kim Pham Ho[1,2], Craig Dahlgren[3], Scott Martin[1], Tanya Kamerman[1,4], and Andy Stamper[1]

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Elkhorn coral (Acropora palmata) and staghorn coral (A. cervicornis) were once the primary reef building corals in the Caribbean, but their populations have declined approximately 98% since the 1980s. In an effort to rehabilitate Caribbean reefs, these corals are commonly grown on nurseries and transplanted back onto decimated reefs. Here, we discuss the methodology for construction of a line nursery at Disney's Castaway Cay®. Since 2011, biologists have conducted semi-annual trips to the Bahamas to test and maintain two nurseries. Early designs encountered entanglement from storm surges, however improvements over the years have resulted in increased durability, successfully withstanding multiple hurricane seasons. Given the cost of labor and travel time, the nurseries were designed to only require a twice-a-year maintenance. Successful growth and transplantation of fragments back onto reefs highlight the productivity of this design.

The Global Coral Restoration Project: Scientists, Conservationists, and Public Aquaria Partner to Rehabilitate Coral Reefs

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SECORE International, The Nature Conservancy, and the California Academy of Sciences have joined forces to tackle the unprecedented decline of coral reefs worldwide. The Global Coral Restoration Project aims to study and scale up coral restoration techniques and practices that have been successful at smaller scales, while integrating coordinated conservation, education, and outreach efforts. Multiple restoration techniques are being used, including sexual coral restoration which has the potential to produce huge numbers of coral offspring from one coral spawning event.

By "seeding" reefs with sexually reproduced coral offspring, we seek to maintain corals' genetic diversity to maximize their ability to adapt to future conditions. Phase one of the project focuses on the Caribbean and includes training for restoration practitioners from coastal communities. Coral husbandry experts and volunteers from public zoos and aquaria play an integral role in conducting trainings, and developing the technology and methods necessary to upscale these techniques.

Exploring a Certification Program for the Marine Aquarium Industry

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The collection of aquarium animals often sees unsustainable capture, environmental damage, high mortality, and extreme economic disparity within the supply chain. As these issues become popularized, it negatively impacts the reputation of the industry, including public aquariums. Our proposed solution is to create a certification program which would provide environmental and social impact information to consumers. By certifying those who follow best practices, consumers can make educated purchasing choices and create market demand for sustainably and ethically sourced animals. Our research has analyzed the previous program by the Marine Aquarium Council and identified structural, strategic, and economic shortcomings. Based on our results, we are developing solutions to the aforementioned issues and are exploring how a revised program could shift the industry toward more sustainable practices. Such a program would have positive implications for public aquariums' sustainability objectives, and their support would be instrumental in raising awareness for these conservation initiatives.

Sponsor: TJP Engineering
Sponsor: Piscene Energetics

Session 3: Shark Conservation
Moderator: Dr. Kathy Heym

Sponsor: 1-2-1 Animal Handling Products

Determining *In Situ* Habitat Use and Migration Patterns of Sand Tiger Sharks (*Carcharias taurus*) in the Western North Atlantic

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Sand tiger shark (*Carcharias taurus*) populations have declined globally, and this shark species is designated as 'Vulnerable' internationally and as a 'Species of Concern' nationally. Despite conservation concerns, little is known about what types of habitat sand tiger sharks require for key life functions, such as foraging and reproducing, necessary for species recovery. To fill knowledge gaps in our understanding of sand tiger shark habitat use and associated migration patterns in the Western North Atlantic, the NC Aquariums, the South-East Zoo Alliance for Reproduction & Conservation (SEZARC), and SEZARC members have formed a team to conduct *in-situ* sand tiger shark research. Our research approach includes three main components: 1) acoustically tagging and tracking sand tiger sharks, 2) remotely collecting video footage of sand tiger sharks, and 3) developing a citizen-science photo identification program. Our *in-situ* efforts complement *ex-situ* research being conducted by zoos and aquaria to conserve sand tiger sharks.

Sand Tiger (Carcharias Taurus) Shark Plasma Testosterone and Semen Seasonality for In Situ and Ex Situ Sharks

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Despite a long history of husbandry for sand tiger sharks, reproduction in aquaria has been largely unsuccessful. To understand why, and as part of a large scale collaborative effort by

aquariums, plasma testosterone and semen were examined from mature ex situ sharks (n=18) January-December, and in situ sharks (n=27) April-August. Plasma testosterone was elevated during winter and spring for ex situ sharks and fell precipitously in summer, remaining low throughout fall, supporting an annual reproductive cycle with spring seasonality. For spring samples, ex situ sharks exposed to natural seawater, light and temperature cycles had higher plasma testosterone than sharks not exposed to those parameters. A significant difference (P>0.01) in sperm motility for semen collected in spring was observed for in situ versus ex situ sand tiger sharks, and 100% of ejaculates from in situ sharks contained motile sperm compared to only 50% from ex situ sharks.

Artificial Insemination of Whitespotted Bamboo *(Chiloscylium plagiosum)* sharks: Raw Versus Seawater Diluted Cold-Stored Semen

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Artificial insemination (AI) using cold-stored semen is a method gaining traction for managing ex situ populations of sharks. Bamboo sharks were inseminated with 100 ul raw (n=1 female), 200 ul 1:1 seawater diluted or 1000 ul 1:10 seawater diluted (n=1 female) semen that was shipped overnight at ~4C from a male at another institution. The female receiving raw semen laid 19 eggs with 12 young successfully hatched. The females receiving diluted semen had 1 young hatch each out of 18 or 15 eggs, respectively. Data from this trial indicate that AI with cold-stored semen collected from bamboo sharks at separate institutions can be used to produce significant numbers of offspring, facilitating movement of genetic material rather than fish to maintain genetically diverse shark populations.

Sponsor: Dr Rob Jones, The Aquarium Vet

Session 4: Shark Conservation and Partnerships
Moderator: Eric Hovland

Sponsor: Tracks Software

AZA SAFE: Sharks and Rays – Accomplishments, Plans, and Getting YOU Involved

Beth Firchau[1] and Hap Fatzinger[2]

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The official Association of Zoos and Aquariums (AZA) describes the Saving Animals From Extinction (SAFE) program as an effort that, "focuses the collective expertise within our accredited zoos and aquariums and leverages their massive audiences to save species. At the same time, SAFE [builds] capacity to increase direct conservation spending, as well as our members' impact on saving species through work in the field, in our zoos and aquariums, and through public engagement.' The SAFE Sharks and Rays Project is one of many projects developed to do just that. Project coordinators, Beth Firchau and Hap Fatzinger, will provide a history of the SAFE effort in our community, advances in the last year, benchmarks for the coming year, and ways that individuals and institutions can participate, collaborate and make an impact on global shark and ray conservation.

Help us help you: Creating Animal Care Manuals for AZA SAFE Sharks and Rays

Kelli Cadenas[1] and Paula Carlson[2]

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The Association of Zoos and Aquariums SAFE (Saving Animals From Extinction) Sharks and Rays program is a challenge because of the large range of taxa it covers. Because it is such a huge undertaking, the SAFE program leaders have created three projects to help jump start the program. Each project is designed to lead to better care, research, and conservation of sharks and rays around the world. Our program is the creation of AZA Animal Care Manuals (ACM) for several key species of sharks and rays. This talk will cover the species that we are working on, how the project is structured, what the requirements for AZA ACM creation is, and how anyone who is interested can get involved. This project is a great way for young professionals in the Public Aquarium industry to get involved in an exciting AZA project.

AZA SAFE: Sharks and Rays – International Elasmobranch Census

Alan Henningsen and Jennie Janssen
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As part of the AZA SAFE: Sharks and Rays project, an initiative was launched in November 2017 to update the International Elasmobranch Census, formerly coordinated by the American Elasmobranch Society. Beyond updating the composition of elasmobranchs in human care world-wide and developing a mechanism for maintaining current data, the co-leaders of the project also aim to determine key information that supports better knowledge of those animals and facilitates communication among care givers. First steps include recruiting Regional Collaborators, developing a format and vehicle for the census, confirming accuracy of taxonomy, and clearly defining "in human care" as it pertains to the census prior to rollout. Participation by Regional Collaborators and all elasmobranch stakeholders will be critical to attain the global reach and impact this project requires.

AZA SAFE – Elasmobranch Phlebotomy and Blood Chemistry Registry

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The Elasmobranch Phlebotomy and Blood Chemistry Registry was initiated in November 2017 to support the AZA SAFE: Sharks and Rays project. Co-leaders were selected for expertise and experience in elasmobranch blood analysis and health assessment. This 3-year project aims to develop best practice guidelines and data references for those working in husbandry, veterinary, commercial laboratory, and research fields. Key topics will cover phlebotomy (blood collection and sample handling), laboratory analysis (cell nomenclature, methodologies and impacts of), and data interpretation. A global database of samples will be compiled based on completeness of the clinical information. The first steps include recruiting collaborators with expertise to advise the leaders, defining the project framework, and organizing a call for blood data. Reference intervals will be determined for species with sufficient sample numbers based on the published standard methods. Outcomes from this project will support elasmobranch care, welfare, and conservation efforts.

Sponsor: Pecan Grove Solutions

Wednesday, May 16th

Session 5: Conservation and Partnerships

Moderator: Ramon Villaverde

Welcome: Andy Wood
Sponsor: McRoberts Sales Co., Inc.

International Sawfish Day - Raising the Bar

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The first International Sawfish Day, dedicated to increasing awareness of sawfish, was held on October 17, 2017. This annual event was created in partnership with the Sawfish Conservation Society (SCS), and the European and American Associations of Zoos and Aquariums. More than 50 participating organizations from around the world including public aquaria, conservation agencies and research facilities held events, shared messages and images on social media, and otherwise spread the word about these amazing animals. Fund raising efforts by The Deep, SCS and others contributed to sawfish field programs in the Sudan. Plans have already begun for next year's event and this momentum will be carried forth in future years solidifying efforts to

strengthen both in situ and ex situ conservation and research efforts, with the intent that along with increased awareness about sawfish, these actions will generate additional funding for these important projects.

Flexin' our Mussels! Freshwater Mussel Conservation

Mikaela Foust
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The National Mississippi River Museum and Aquarium (NMRMA) has a unique partnership with the U.S Fish and Wildlife Service to assist in the propagation of endangered freshwater mussels and enabling us to create a dynamic citizen science program. Mussels are considered one of the most imperiled taxa in North America. NMRMA has been involved in mussel propagation since 2010. In an effort to expand our partnership and engage more visitors our project grew to 16 Submersible Upwelling System (SUSPY) buckets by the spring of 2017. Within two years of the SUSPY program, >900 students helped to monitor and partake in the captive propagation of endangered species, such as the Higgins eye pearly mussel. This year NMRMA expanded to add a permanent, live, mussel exhibit and a conservation lab to propagate Logperch so USFWS can increase propagation of the endangered Snuffbox Mussel.

Collaborative Efforts to Save the Endangered Giant Sea Bass

Nicole Leier
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The giant sea bass (Stereolepis gigas) is a critically endangered species off the coast of California. Current wild population estimates are as low as 500 individuals. In 2016, the Aquarium of the Pacific was the first aquarium to successfully rear a giant sea bass. Aquariums, universities, aquaculture facilities and government agencies conducting research on this species were used as resources to aid in the success of raising this individual. During this collaborative process, it was suggested that giant sea bass experts come together in order to share knowledge for the benefit of this species. The Aquarium of the Pacific decided to host the first-ever Giant Sea Bass Symposium. This presentation will give a synopsis of the current research on this species and discuss how the Aquarium of the Pacific is actively involved in helping researchers.

Developing Fisheries to Save the Nautiluses? The Nautilus Strong Initiative

Gregory Jeff Barord, PhD
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Nautilus exports are now regulated by the Convention on International Trade in Endangered Species (CITES) and require a permit showing the export does not negatively affect populations. Although this permitting process has effectively curtailed legal exports, particularly

for educational purposes at public zoos and aquariums, local unregulated fisheries and trade persist while the potential of an international black-market system looms. Thus, developing a management system for nautilus fisheries is essential to protect nautiluses and support the CITES mandates. Through the Nautilus Strong Initiative, fishery takes and exports will be traceable and provide data to monitor population trends. This also provides a clear chain of custody for nautilus acquisitions and makes it possible for educational institutions to acquire specific sex ratios, ages, and species, for the first time. Now, more than ever, the continued display of nautiluses and accompanied educational messages are critical towards effectively saving the nautiluses.

Seattle Aquarium Reef Surveys:
Contributing to the Management of Temperate and Tropical Fish

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Since the mid-2000s, the Seattle Aquarium has conducted SCUBA-diver-facilitated video transect studies on reefs in Washington and Hawaii. These non-invasive studies produce a permanent record of the baseline populations of local fish and invertebrates over time, and have contributed to the data used to manage and regulate fisheries. In Washington, increasing concern over the long-term stability of bottomfish populations motivated the Aquarium to begin formalized monitoring in 2005. Reef fish populations in Hawaii are a major concern for both food and marine ornamental industries, and overall coral reef health; the Seattle Aquarium chose to undertake an active conservation role in Hawaii and initiated annual video transects there in 2009. Methods used for these studies are based on techniques similar to those established by Drs. Brian Tissot and Bill Walsh, and produce repeatable annual measurements of fish and invertebrate abundance, to quantify changes in species diversity and population levels over time.

Sponsor: See Clear

Session 6: Conservation & Partnerships: Panel Discussion
Moderator/Facilitator: Paula Carlson, *paula@dwazoo.com*

Sponsor: Dynasty Marine Associates, Inc.

Panel Discussion: Strategic Partnering to Support Our Common Goals

Amy Tillman (The Florida Aquarium) Atillman@flaquarium.org; Tim Carpenter (Seattle Aquarium) T.Carpenter@seattleaquarium.org; Sandy Moore (Segrest Farms, Pet Industry Joint Advisory Council) sandy.moore@segrestfarms.com; Dr Rob Jones (Aquarium Vet) rob@theaquariumvet.com; Beth Firchau (MFTAG Chair, AAC Vice Chair, SAFE Sharks and Ray SSP Sustainability Project Manager) bfirchau@audubоninstitute.org; Scott Dowd (New England Aquarium, Project Piaba) sdowd@neaq.org; Jacqueline Anderson (New England Aquarium) Jacqueline.anderson@neaq.org; Martin Moe (Retired Marine Biologist, Marine Aquaculture) martin_moe@yahoo.com

Regulations, legislation, community- and cultural- based environmental movements, and the complexities of animal welfare and animal rights conversations all affect the home and public aquarium industries and their common mission of promoting the thoughtful and responsible

stewardship of our aquatic resources. A two-panel discussion will review recent lessons learned and ongoing homework required on the front lines of one facet of the public and home aquarium industries – animal acquisition and share the importance of considering the impacts of our acquisition policies on the communities and cultures at the epicenter of our resources. Take homes will include a call to create engagement opportunities with untraditional partners, how to engage in and elevate acquisition sourcing conversations in facilities, and tools and resources available to ensure responsible practices.

Session 7: Guest Programs
Moderator: Shawn Garner

Sponsor: US Mysids

Shark Talk – How to Train, Care for, Protect and Educate

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Dolphin Quest Hawaii temporarily acquired two blacktip reef sharks in 2013 from an oceanographic school as educational ambassadors for their wild counterparts. Their habitat viewing was free and open to the public giving guests a unique opportunity to learn about them in a way they may not have otherwise had. During a daily Shark Talk we coupled trained behaviors with conservation messaging allowing us to dispel many of the myths and misconceptions guests had about sharks. Guests also learned about threats sharks face in the wild and what they could do to help them. In preparation for their return back to the school, we trained an innovative, voluntary collection for one of the sharks. Our continued involvement with the school is helping to teach the next generation about the importance of sharks, bridging the gap in conservation management and how to enhance the wellness of animals under human care.

The Other Side of the Acrylic: Taking Guest Interaction to a New Level

Denise Swider and Gretchen Scrimger
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Discovery Cove is an interactive resort-style zoological park. Since its inception in 2000, Discovery Cove has been immersing guests into animal environments achieving its mission to inspire people to appreciate and care for animals, both in the park and around the world. In 2017, Discovery Cove added two new guest interactive programs. “Ray Feeding” creates an opportunity for guests to join the aquarists for a morning ray feeding followed by a guided swim in the Grand Reef. “Shark Swim” allows a limited number of guests to participate in a shark training session, learn about shark behavior, physiology, and conservation and freely swim with multiple shark species. Developing and implementing two new revenue-generating guest interactive programs in the span of four months proved to be an exciting and challenging undertaking. Experienced shark

personnel and the collaborative efforts of multiple departments facilitated a safe and successful launch for both programs.

Her Name is Lola: Incorporating a Prosthetic *Lepidochelys kempii* Flipper Research Project into Contemporary Rehabilitation Practices and Public Education Programs

Genya Yerkes
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The purpose of this project is rehabilitation via prosthesis and contemporary behavioral conditioning techniques, enabling a "powerstroke" that was previously unattainable. This project has also concurrently supported a heightened level of public awareness in supporting conservation, as called for in sections I-36 to I-38 in the Bi-National Recovery Plan. Guests observe us fit the prosthesis daily, which was created by PhD candidates at Worchester Polytechnic University with carapace dimensions, x-rays, MRI scans, and data provided by KWAQ. By incorporating the daily task of fitting the apparatus onto the amputee into our daily "Sea Turtle Conservation" talk, guests have a real-time experience with an active research project. It has greatly increased our ability to effectively educate the public. Through attendance data, guest surveys, and veterinary feedback we have concluded that these areas have benefitted from the prosthesis project: Conservation Goals/Quality of Life/Research Data/Future positive impacts on chelonian amputees /Public Outreach/Media Coverage

Going Viral: The Role of Social Media in the Aquarist Industry

Angelina Komatovich
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As aquarists, we create exhibits that tell stories. However, we spend most of our time behind the scenes, hidden from public view. The Aquarium of the Pacific has created a new senior aquarist responsibility titled Social Media Liaison. This role provides a conduit for increased communication between departments to better support the Aquarium's mission. The liaison is responsible for facilitating interesting information to the development, marketing, and education departments. This new position greatly increases the timeliness and exposure of stories regarding the critical nature of the aquarist's role in animal care. Social media can be used as a tool to promote conservation, research, recruit volunteers/interns, inspire donors, and highlight the exceptional care we provide our animals. Through this internal collaboration, we extend our reach beyond aquarium visitors, providing the global public with a deeper sense of wonder, respect, and stewardship for the Pacific Ocean, its inhabitants, and ecosystems.

Session 8: Workforce Development
Moderator: Laura Wandel

Sponsor: Tenji, Inc.

"You've Got Herps: An Aquarist's Journey into the World of Reptiles and Amphibians."

Rebecca Bray
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What happens when you've spent your whole career working with fish and you're suddenly handed reptiles to care for? Professional development. As aquarium collections diversify into the terrestrial realm, there is a potential for employee development and enrichment. This talk will explore these potentials for development and how the skills that you already have as an aquarist can prepare you for working with species you may have never thought you would work with.

Built by Aquarist for Aquarist: The Creation and Implementation of a Curriculum Based Internship Program

Johnny May and Joleena Jewell
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We all know this industry is a competitive industry to start a career in. For many, some form of volunteer or internship work allowed us to get where we are today. Looking back, there were many things we learned from our early experiences and there are many more things we wish we had been exposed to prior to landing our first jobs as professional aquarist. OdySea Aquarium opened in September of 2016. With over 2,000,000 million gallons of exhibits and over 40 animal care professionals on staff, we knew endless learning opportunities for passionate individuals existed. The purpose of this presentation is to discuss the challenges associated in developing a successful internship program from the ground up at a for-profit facility. Topics to be discussed include legal and financial considerations, recruitment, program design along with a multitude of other considerations that must be made prior to starting an internship program.

Aquatic Systems Management Issues

Juan Sabalones
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Aquatic systems are defined as: 1. Aquariums 2. Aquatic exhibits with aquatic animals other than fish and invertebrates 3. Water Features 4. Combinations of the above. Institutions without the benefit of a dedicated Life Support System (LSS) section are often challenged when faced with LSS issues for their systems. This presentation will present and review options available to such institutions in terms of seeking help with problem solving and staff training.

National Aquarium Standard Development in China

Xianfeng Zhang
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There are about 200 public aquariums currently in China. The number of aquariums has been increasingly growing from mid-1990 through now. This highlights the inconsistency between the needs of animal welfare and the requirements of aquarium construction and management. Two systems of aquarium standards, mainly for marine mammals, have been developing since early 2000. One system is for marine mammal trainer regulation, which is called "National Vocation Standards for Marine Mammal Trainer" issued by Ministry of Labor and Social Security and began to take effect in 2005. Another is for aquarium construction and animal wellness, which includes series standards, such as requirements for facility design, water quality, studbook keeping, animal husbandry, etc. Parts of the standards had been issued by Ministry of Agriculture and began to take effect in 2013, and parts of them are being studied and will be issued soon. Here we show the story how the standards developed.

Sponsor: Aqua-Tech Co.

Thursday, May 17th

Session 9: Animal Behavior 1

Moderator: Maegan Gentry

Welcome: Dr. Kathy Heym

Sponsor: Asahi/America

Juggling Cats: Coordinating the Daily "Reef Feed" at The Florida Aquarium

Laura Wandel and Jenny McAndrews
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The 'Coral Reef' is the largest habitat at The Florida Aquarium with a volume of approximately 500,000 gallons. The habitat is home to 74 distinct species and is used throughout the day for public dive programs. Ensuring that every animal gets the proper nutrition is a challenge all on its own, but FLAQ is committed to providing individualized care and husbandry/medical related training to as many animals as possible. Currently the 'Coral Reef' contains more than 2 dozen individual animals in 8 separate species groups that receive specialized care and training during a single daily training session. This presentation will discuss how all of these groups are coordinated in concert with each other and the individual group challenges and successes of the Reef Feed training program.

Improving Animal Wellness in Southern Stingrays (*Dasyatis americana*) through Behavioral Modification

Jessica Sandelli and Alyssa Fessett
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The Florida Aquarium houses seven female Southern Rays in the deep side of our 500,000-gallon coral reef habitat. This habitat contains most of the aquarium's elasmobranch collection within either the shallow or deep sides separated by a corralled platform. In this environment, we developed a training plan for the rays that has improved the time of catchups, reduced staff and animal stress, and ensured all samples are collected on schedule in preparation for sampling with the South-East Zoo Alliance for Reproduction and Conservation (SEZARC).

By inviting the Southern Rays into the corral, we condensed the time needed to move the rays while decreasing the amount of staff required through reducing the need to dive. Six out the seven Southern Rays came into the corral when invited; divers brought in the last.

Moving forward, we hope to add behaviors to the rays' repertoire resulting in increased participation in wellness assessments.

The Taming of the Shrew – Charlotte's Story

Stephanie Shannon
Mystic Aquarium
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When you've got a Green Sea Turtle on a 100 day hunger strike and you need to get her to participate in her own health care… what do you do? This talk will go into the tactics used to condition our ~20 y/o Sea Turtle to help her progress from despondent to willingly stationing on a platform under UV light. The talk will also go into the animal's history/rehabilitation and weight pouches used to compensate for the positive buoyancy "Bubble Butt" caused by the accident and how it affected training. *Spoiler Alert*Also turns out Charlotte is really a Charlie…

Session 10: Animal Behavior 2
Moderator: Aaron Jeskie

Keeping Fit; Healthy Body, Mind, Alligator and Penguin

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As our industry grows and evolves, our techniques, attitudes, and animal welfare advances as well. Training our animals, aquatics and terrestrials alike, has become a significant part of our daily routines. This training allows us to provide the creatures in our care with mental and physical

stimulation, reduced stress during physicals, ease of catch-up, as well as improved relationships between animals and trainers. At the Florida Aquarium, the animal welfare programs for two of our semi-aquatic animals, American alligators and African black-footed penguins, allow for the best care and comfort of our animals. The behaviors we have taught our American alligators and African penguins also provide our biologists with ease of care, ease of medical exams and exhibit maintenance, as well as fun, engaging, and connective experiences for our guests. It is hoped that this presentation inspires other animal care professionals with training techniques and ideas for their animals.

Methods for Stretcher Training the Bonnethead Shark *(Sphyrna tiburo)*

Hannah Cutting
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Training of elasmobranchs in public aquariums is an emerging husbandry practice used to improve overall care and reduce stress associated with handling. In 2016, animal care staff at the New England Aquarium in Boston, Massachusetts made the decision to begin stretcher training the bonnethead shark, the only shark species in our main exhibit, the Giant Ocean Tank (GOT). Prior to training, the sharks were stick-fed by divers, which presented many challenges, including risks to the divers due to the sharks' excited and unpredictable response while feeding. Training took place at the NEAq's offsite facility in a 30' round tank containing six bonnethead sharks. The sharks' behaviors were gradually modified to train them to consistently follow a target into a stretcher for feeding. Three sharks have been successfully transitioned back into the GOT using this method. This presentation demonstrates the methods for offsite training as well as keys to a successful transition back onto exhibit.

Cleatus: Working with a Goliath Grouper

Laura Wandel and Tori Davis
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Cleatus has made his home at the Florida Aquarium since 1995 and in recent years he has participated in a training and enrichment program dedicated solely to him. The past 2 years have seen large periods (60+ days) of inappetence during the late summer/fall season with reduced participation in training and enrichment sessions. This presentation will detail the variety of methods used to encourage him to eat as well as the subsequent behavioral conditioning put in place to make future handling and veterinary procedures less stressful for Cleatus and the husbandry team.

Blood in the Water

Chris Schreiber
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The Georgia Aquarium's animal care team took on the task of rethinking how to go about minimizing stress on whale sharks in its care, yet still have the ability to acquire blood samples for

analysis. Previous efforts required a large team that steered the shark into a vinyl stretcher to lift the shark's tail out of the water. This new effort would again require the entire team's participation as well as support from other departments and would occur during a normal feeding session. A desensitization process preceded each attempt that allowed those feeding, the divers, and the sharks to become familiar with each other. Utilizing knowledge gleaned from previous attempts on one particular specimen, the team successfully obtained blood samples from the other three whale sharks on the first attempt each time. Additionally, documentation of this process was prioritized which would allow it to be repeated and even improved upon.

Session 11: RAW Business Meeting (open)
Jeff Gibula, RAW Advisory Committee Chair

General RAW Business
RAW 2020 Bid Presentations
RAW 2019 - Columbus Zoo & Aquarium Update and Propaganda

Session 12: Veterinary Care 1
Moderator: Dr Ari Fustukjian

Sponsor: Animal Necessity

Experience Treating Closed Systems with Chloroquine Phosphate and the Resulting Potential Side Effects.

Kelly Sowers[1] and Leah Neal[2]

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[2]Ripley's Aquarium of Canada, 288 Bremner Blvd., Toronto, ON M5V 3L9

The use of chloroquine phosphate as a chemical treatment for saltwater aquaria can be implemented for a variety of medicinal factors. Having used this treatment for elasmobranch, as well as mixed-species systems, differing effects on the animals both during and after treatment were noted. Based on the resulting effects, byproducts produced during the removal process may have a greater noted impact than the drug itself, both in behavior as well as mortality.

The Red Queen: Using Hyposalinity to Treat *Neobenedenia* sp. and *Cryptocaryon irritans* in a Large Multi-Taxa Exhibit

Mark Smith, Charlie Innis, Nina Fischer, Barbara Bailey, Steve Bailey, Chris Fernald, Dan Laughlin, and Mike O'Neill
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Giant Ocean Tank (GOT) is the central multi-species exhibit at the New England Aquarium, with a volume of 850 m^3 (225,000 gal), and a diverse population (~1,000 individuals; ~100 species) of tropical Caribbean reef fishes. In 2016, *Neobenedenia* sp. was diagnosed in the GOT. Praziquantel treatment was administered nine times over a two-month period, each dose targeting an initial concentration of 4 mg/L. Presence of *Neobenedenia* was monitored by

examinations of live fishes, sampling during necropsies, and two 64 x 32 cm, 100 µm mesh panels, installed in GOT surface skimmers to trap egg capsules. During praziquantel treatment, copper sulfate also was administered twice (for two weeks and four weeks, respectively), conservatively at 0.06 mg/L, due to a parallel outbreak of *Cryptocaryon irritans*. Presence of *C. irritans* was monitored using a qualitative measure of fish health, referred to as disease condition status (DCS). During chemotherapeutic treatment, mortality rate in the exhibit was almost a magnitude higher (n = 59 / month) than normal operating conditions. Presence of *C. irritans* abated, but *Neobenedenia* persisted. Over the next 12 months, hyposalinity was used to treat *Neobenedenia*. Salinity was reduced from 32 g/L to 15 g/L for ~60 days, then raised to and maintained at 22 g/L as a prophylactic measure. A brief outbreak of *C. irritans* was observed nine months after treatment commenced, but was managed by dropping salinity back to 15 g/L (for ~60 days) and then returning it to 22 g/L. No adult *Neobenedenia* were detected after hyposalinity treatment commenced. *Neobenedenia* egg capsules were detected, in slowly declining numbers, for the first nine months of hyposalinity treatment, but have not been detected for three months (at time of writing). Mortality rate during hyposalinity treatment was similar to that seen during non-disease periods (n = 7 / month).

Developmental Life Cycle of Marine Monogeneans (*Decacotyle* sp.) on Aquarium Housed White-Spotted Eagle Rays

Nancy Kim Pham Ho[1,2], Britney Mosey[1,2,3], Elizabeth Nolan[1], and Andy Stamper[1]

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[3]Sea Life Minnesota Aquarium at Mall of America, Mall of America, East Side Level 1, 120 East Broadway, Bloomington, MN 55425
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Marine monogeneans (*Decacotyle* sp.) frequently parasitize the gills of white-spotted eagle rays and can be detrimental to the welfare of the animal. Management of these parasites often includes medication with praziquantel baths which provide temporary relief, but may not fully eradicate the parasite population. One reason is praziquantel's susceptibility to microbial degradation which may cause erratic concentrations within a system. Additionally, since eggs are refectory to many therapeutics, a series of treatments or management strategies may need to be employed to prevent all life stages of the parasite from infesting the rays. This presentation will highlight various practices employed at The Seas, Epcot®, Walt Disney World® Resorts, including information on the development and response of *Decacotyle* egg hatching rates and activity responses of adult monogeneans to various therapeutic treatments.

Pain in Fish - Fact or Fiction?

Dr. Rob Jones
The Aquarium Vet, PO Box 2327, Moorabbin, Victoria 3189, Australia
rob@theaquariumvet.com

Fish welfare is an increasingly important subject. We need to understand what it is and why it is so important to be concerned about the welfare of the fish under our care. As an industry we will increasingly come under scrutiny from various animal rights organizations. An increasingly important part of this discussion is the question "Do Fish Feel Pain?" This has been a contentious issue for the past decade. We will examine this question from a scientific approach, and attempt to resolve this issue once and for all.

Session 13: Veterinary Care 2 and Nutrition
Moderator: Rachel Serafin

Sponsor: Species 360

Utilizing 3D Printing as an Alternative Method to Compensate for Buoyancy Imbalance Issues with *Chelonia mydas* in Public Aquariums

Traner Knott
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The use of weights to improve the buoyancy and swimming position of boat strike sea turtles is not a new concept in zoos and aquariums. However, the main methods of affixing weights (epoxy directly applied to the carapace or custom-built nylon harnesses with weight pockets) have drawbacks including long application processes, entrapment risks and inconsistent adhesion success rates.

SEA LIFE Minnesota has a 100-pound rescued Green sea turtle. The sea turtle suffered a boat strike with significant damage to its carapace. Supplemental weights are needed to avoid positive buoyancy.

SEA LIFE Minnesota and the University of Minnesota have recently developed an alternative approach using 3D printing. The "Exo-Shell" will provide a more reliable attachment point and flexibility for rapid buoyancy adjustment. The shell will also minimize the risk of entanglement and adhesion failure while allowing real time weight adjustment without lengthy dry dock sessions.

Pooling Our Resources: Using Global Data to Create Invaluable Animal Care Tools

Meredith Knott
Species360
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ZIMS is the most widely used records system within the zoo and aquarium community. Partnering with the Institute of Museum and Library Sciences, Species360 focused on extracting, summarizing and organizing information contained within millions of medical records to produce resources that could support and improve veterinary care in aquariums and zoos. The project

successfully produced 3 completely new medical resources (Anesthesia Summaries, Drug Usage Extracts, Morbidity and Mortality Analysis) and significantly enhanced an existing resource (Expected Test Results). Each resource provides easy, searchable access to a unique compilation of medical experience and knowledge that is useful to animal care staff and conservation research partners. As aquariums increasingly pursue in-situ and ex-situ conservation goals, the community need for data sharing and pooled resources grows exponentially each year. Acknowledgements: This project was supported by the Institute of Museum and Library Services grant MG-30-14-0039-14.

Imuno-2865® Supplementation and its Effects on Blood Parameters in the Sea Bream (*Sparus aurata* Linnaeus, 1758) in Aquaculture

Ivan Župan[1*], Suzana Tkalčić[2](presenter), Tomislav Šarić[1], Rozalindra Čož-Rakovac[3], Ivančica Strunjak-Perović[3], Natalija Topić-Popović[3], Nina Poljičak-Milas[4], Matko Kardum[4], Danijel Kanski[5], Tomislav Bulat[6], Blanka Beer Ljubić[7], Vesna Matijatko[7]

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[5]PhD candidate at University of Zagreb, Faculty of Agronomy, 10000 Zagreb, Croatia
[6]DALMAR, Pakoštane, 23000, Croatia
[7]University of Zagreb, Faculty of Veterinary Medicine, Internal Diseases Clinic, 10000 Zagreb, Croatia
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Many prophylactic dietary supplements are being considered for aquaculture fish diet. We studied in vivo effects of IMUNO-2865®, a natural hemicellulose compound, on various hematological and oxidative stress parameters in farmed sea bream under winter conditions. A 6-month research included 640 sea bream (18 month-old, 277.8± 38.0g), divided into 4 groups and fed commercial pelleted food mixed with 0g, 1g, 10g, and 25g of IMUNO-2865®/kg feed. Samples were collected on days 0, 30, 60, 90 (final feeding), and 180. Most cellular blood parameters fluctuated between sampling periods, but a significant increase in Ca++ levels, total protein and NH3, monocyte, erythrocyte, and heterophil numbers were observed in Group 4. Superoxide dismutase, glutathione peroxidase, and serum paraoxonase were also significantly increased in groups 3 and 4 in later phases of the experiment. Presented data and lack of mortality suggest an overall safe, positive and potentially immunostimulative effect of IMUNO-2865® in seabream aquaculture.

From Cyst to Adult: Low-Budget Hacks for In-House *Artemia salina* Cultures

Morgan A. Lindemayer-Finck
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The common brine shrimp, *Artemia salina*, have been utilized as a live food in the aquarium industry since the 1920s. They provide a quality high protein diet leading to the greater survival rates, faster growth rates and fuller color development of their consumers. Many institutions dedicate financial resources on ordering live Artemia to support juvenile fishes or high maintenance species. The New England Aquarium has allocated this expense into successfully creating an in-house culture that sustains the institution using a low-cost, low-maintenance design incorporating hatching cones, grow-out barrels, and cold storage methods. Not only has this culture served to put money back into the aquarium and increase our sustainability, it also allows for a healthier product. Having control over the culture conditions of our live foods gives us more control over what we are adding to our tanks and feeding our fishes, leading to a more successful and viable collection.

Session 14: Animal Handling
Moderator: Stephen Schwanebeck

Sponsor: Kessil

Safe Handling of Stingrays

Dr. Rob Jones[1] and Clem Kouijzer[2]

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One of the greatest Health and Safety issues in public aquariums is the safe handling of stingrays. There have been many serious injuries including one fatality in public aquariums.
The anatomy of the stingray barb and its venom will be presented. We will examine a variety of safe handling techniques and personal protection equipment (PPE) as well a prototype device designed to cover the barb during handling and to protect aquarists.

Steel, Plastic and Mesh: DIY Nets and Traps

Chris Okamoto
Cabrillo Marine Aquarium
chris.okamoto@lacity.org

Aquarists often work in different environments collecting different organisms and having the right equipment saves valuable time. With the various needs a facility has for different sizes and shapes of nets and traps, the proper one may not be available commercially. The ability to

make custom collection gear (exact to specific requirements) can help ensure safe capture and transfer of animals. Custom nets and traps are often very expensive but with a little ingenuity, you can build your own out of fairly inexpensive materials to suit your needs. The Cabrillo Marine Aquarium will go over the basics as well as the sources we use to create some of our own custom nets and traps.

Friday, May 18th

Session 15:

Moderator:

Welcome: Ron Pendergrass
Sponsor: Aquatic Exhibits International

Survivin‘ and Thrivin‘ after Cat. Fivin‘

Caroline Emch-Wei and Ryan Firment
Coral World Ocean Park, 6450 Coki Point, St. Thomas, VI, 00802
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It’s rare to be hit by a hurricane of any kind in St. Thomas, let alone two back to back category 5 hurricanes. Yet in September 2017, Coral World Ocean Park sustained heavy damage from both Irma and Maria and left the aquarists with unique problems we needed to solve to keep our animals alive and get the aquarium open to the public again. As an island we were left without power for months and limited food options due to damaged shipping ports in Florida and throughout the Caribbean. At Coral World we endured damaged water lines, damaged exhibits and missing roofs, limited staff, and limited animal housing. We learned the importance of generators, rationing, water conservation, construction with limited tools, cross training, and creative animal transport and housing options. These lessons, taught the hard way to us, can passed on the easy way to you at RAW.

Six Years in the Making: The Path to an Effective Autotrophic Denitrification System

Kyle McPheeters
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Autotrophic denitrification is a means of filtration that reduces nitrate without water changes, giving an inland aquarium the ability to improve the health of marine exhibits without the environmental and fiscal costs of changing synthetic seawater. Over six years four unique denitrification systems were constructed. The merits of each type of system include ease of media management through backwashing, consistent flow rates/performance with predictable results, and lower nitrates in the exhibit; shortfalls include inconsistent results, unpredictable performance, difficulty managing media and difficulty cleaning media. Trials with these systems show that to be effective two separate media vessels (sulfur and carbonate) should be used, and systems should

be recirculating. Our systems and the real-world data produced from them addresses current misinformation.

Successful Denitrification at the National Aquarium - Six Years and Counting

Andy Aiken
National Aquarium
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Long-term exposure to nitrate in excess of 200 mg/L as NO3- is linked to various health problems including goiter in elasmobranchs and depressed antibody response in teleosts. Nitrate management via water changes on large, closed exhibits is often cost-prohibitive and inadequate. Since 2011, autotrophic denitrification has been used successfully at the National Aquarium. Concentrations below 10 mg/L as NO3- have been achieved in multi-taxa exhibits as large as 1,230,000 liters. No custom-built components are used. Process management is user-friendly and consists of daily olfactory inspection, 3×/week nitrite (NO2-) testing of denitrification system effluent; and weekly or monthly nitrate testing of exhibit water via ion chromatography. Automated control and monitoring (e.g. pH, ORP) is not required. Systems are backwashed weekly. Multiple improvements have advanced system reliability and ease of use. Recirculating reverse flow regime through sulfur and calcium carbonate allows daily removal rates greater than 7 kg NO3- /m3 sulfur.

AC / DC – The Advantages of Converting to Direct Current Pumps

Mark Smith and Barbara Bailey
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Traditional alternating current (AC) pool pumps have been used in the aquarium industry for decades. Although AC pumps can accomplish desired flow rates for most small aquarium systems, at a reasonable cost, they can have significant drawbacks, including: need for regular maintenance (e.g., replacement of seals); generation of excessive ambient noise, vibrations, and heat (both into the atmosphere and the water stream); and elevated rates of energy consumption. In late 2016, the New England Aquarium received funding from a Trustee to replace 17 traditional AC pumps in our Animal Care Center Quarantine Room with Abyzz direct current (DC) pumps. DC pumps (200 and 400 W) were installed over a one-year period. The intention of this exercise was to analyze the feasibility of replacing AC pumps (approx. 150) on smaller aquatic systems (exhibits, quarantine systems and holding systems) throughout the Aquarium. The following data sets were collected and compared for AC and DC pumps: energy consumption, cost to operate, noise, vibration, and system temperature changes.

Sponsor: Aquatic Equipment & Design, Inc.

Session 16: Water Quality
Moderator: Becky Ellsworth

Sponsor: Fritz Aquatics

Gram-Negative Bacteria and Its Implications for Aquatic Animal Health

Crystal Gentle
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cgentle@cscmail.org

Public aquaria are regulated by agencies such as USDA and OSHA, and may meet standards of other groups such as AZA and EPA when it comes to microbiological water quality testing. Some gram-negative bacteria are a factor in human health issues and may also contribute to non-human animal issues. Therefore, many institutions go beyond basic compliance tests for the safety of the animals, staff, volunteers, and public. The California Science Center regularly performs microbiological tests on its aquatic systems, however the relevance of the frequency of testing and corresponding response had not been formally established. In order to determine whether there is a correlation between counts of gram-negative bacteria, husbandry practices, and animal health issues, we compared our test results with our daily husbandry and veterinary records to identify and analyze patterns and trends. This information can be used to alter daily husbandry practices in order to maintain animal health.

Laboratory Results at Hobbyist Pricing: Optimizing Water Quality Testing for Accuracy and Efficiency

Chris Emmet
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Monitoring of water quality parameters is critical for the proper husbandry of any aquatic animal. As animal welfare and health are primary goals of any well-run facility, top-of-the-line tests are used to measure these parameters. However, these tests can be time-consuming, expensive, or potentially produce waste that requires special disposal. Despite the downsides, these tests give precise results which are essential in a professional organization. In contrast, hobbyist-grade reagents are a "quick and dirty," solution; less expensive, less precise, but often simpler. I propose that these two methods can be combined, using hobbyist reagents in tandem with proper laboratory technique, and analyzing the resulting color with a spectrophotometer to eliminate imprecision. This process would involve using standard solutions to build a model for individual tests, and then testing with samples of tank water. If successful, this could offer institutions a less-expensive, but still accurate method to measure water quality.

Water Quality for Herptiles; Not Just a Fishy Business

Ryan O'Shea
Tampa's Lowry Park Zoo
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When considering animal health and care, environmental factors should be taken as seriously as the patient. With many reptiles and amphibians we focus on environmental temperatures and humidity to ensure our patients are being maintained at their preferred optimal temperature zone and ideal humidity. For aquatic and amphibious species, water quality should also be considered while collecting environmental data. The following is a collection of recommended and suggested water quality values as they relate to reptilian and amphibian care.

Poster Abstracts

Monitoring Swallow Tailed Kites in the Tampa Bay Area

Jenny McAndrews
The Florida Aquarium
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The Florida Aquarium partners with The Avian Research and Conservation Institute (ARCI) to help support migration studies and annual population monitoring of Swallow-Tailed Kites (*Elanoides forficatus*) in the Tampa Bay Area. Previous research on Swallow Tailed Kite nesting ecology, suggests that roosts should be identified and monitored due to the large decrease in their population in the 1940s. This inspired one of The Florida Aquarium's conservation goals to help monitor this species right in our backyard. From field work, to education at the aquarium, we strive to raise public awareness about the importance of Swallow-Tailed Kites as a keystone species in our area. Thereby, we are able to present how important the Florida ecosystem is to sustaining their population.

From Harbor to Reef: An Hawaiian Coral's Journey Through a Visual Montage

Chan, N.T., Wolke, C.S., Del Rio Torres, L., Gulko, D.A.
Hawaii Coral Restoration Nursery, Hawaii Division of Aquatic Resources

Hawaiian corals collected from low value areas, like harbors, are given a second chance with the Hawaii Coral Nursery's Fast Growth Protocol by increasing their size in a short amount of time for out planting onto impacted reefs. This fast growth process takes a 10 cm coral at collection to a final size of > 40 cm, a process that would typically take 25-30 years in a natural setting given the average Hawaiian growth rate of 1-2 cm/year. A photographic journey of a Hawaiian coral through the Hawaii Coral Nursery has been documented from collection to outplant.

Managing A Nuisance Cichlid Population within a Large Multi-Taxa Exhibit

Jenoh Gonzales
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Cichlids are aggressive, elusive, breed readily and will thus multiply rapidly in large exhibits without predators. This presents problems in collection planning and exhibit carrying capacity, not to mention the impact on exhibit aesthetics and theme. Unfortunately, this became the case with the 25,000-gallon, multi-taxa Southern Swamp Exhibit at the California Academy of Sciences, Steinhart Aquarium. The goal was to remove cichlids so as to not affect or harm other fish, or require removing a 10ft alligator and 5 large snapping turtles. To date we have tried seine nets, minnow traps, ring nets, cast nets, hook and line, butterfly nets and tickle sticks with varying degrees of success. In the end, the use of a Hawaiian sling to spear the fish proves to be the most effective, albeit lethal, method to manage this population. This presentation chronicles our efforts and rationale.

The Central Campus Story: The Past, Present, and Future

Gregory Jeff Barord, PhD and Kirk Embree
Central Campus, Marine Science Department, 1800 Grand Avenue, Des Moines, Iowa 50309
Gregory.barord@dmschools.org

What happens when high school students take control of over 150 species of organisms housed in over 100 marine aquariums? Well, it's complicated, but exciting…!!! In Des Moines, Iowa, 150 new high school students enroll in an Aquarium Science or Marine Biology course at Central Campus Regional Academy, each year. Through feedings, husbandry, and water quality to live food cultures, veterinary treatments, and research projects, students receive opportunities to develop invaluable career skills and knowledge every day. Students are also challenged to leave a legacy for future students that builds upon previous efforts and improves the overall program. Last year, a student designed a novel grass shrimp aquaculture system that increased output, reduced expenses, and improved water quality. This year, students are developing a large-scale reef fish system with the goal of collecting and rearing larval fishes. Central Campus empowers students of today, to become leaders of today, and tomorrow!

A BRIEF GUIDE TO AUTHORS

Updated 2019

This guide is intended for those not accustomed to using a "Guide to Authors", as provided by more formal periodicals. Historically only about 5% of *D&C* authors get this correct ☺. Please help me out folks!

As always, typical Drum & Croaker articles are not peer reviewed and content will not be edited, other than to correct obvious errors, clarify translations, modify incorrect or cumbersome formatting, or delete superfluous material. Other types of contributions (announcements, etc.) may be edited to meet space limitations.

The approximate deadline for submissions is December 15th. As has always been the case, materials in *Drum and Croaker* may be reproduced unless otherwise specified. Please credit *Drum and Croaker* and the contributor. I expect and assume that all submissions to D&C (papers, photographs, etc.) have been authorized by all original authors or co-authors, do not infringe on any copyright or prior publication agreements, and have successfully completed any internal review process required by your institution.

Submit articles via email as a Microsoft Word document (or a file that can be opened in Word). **My E-mail address is petemohan55@gmail.com.**

All Articles Must Adhere to the Following Basic Format:

- Use justified, single-spaced, Times New Roman 12-point font throughout (except for the title section, and figure and table legends as noted below).
- A4 users please reformat to 8 ½ x 11-inch documents (North American "letter").
- Keep the resolution of photographs LOW. High resolution photos make the PDF file huge and are compressed anyway.
- **Format the title section with the line spacing set on 1.5 lines (not another method) and using centered, boldface font. Only the title should be CAPITALIZED (except italicized *Scientific namesii*)**. When using MS Word, go to the "Home" tab, open the detail on the "Paragraph" section, and choose "1.5 lines" under spacing and make sure the before and after spacing settings are at "zero".
- Double-space after your "institution name" to begin the body of your text. When correct the title and headings formatting should look like this:

(continued on next page)

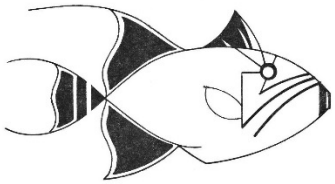
USE OF DUCT TAPE IN THE HUSBANDRY OF *Genus species* AT FISHLAND

Jill Fishhead, Senior Aquarist jfishhead@fishstinking.com

Fishland of South Dakota, 1 Stinking Desert Highway, Badlands, SD, USA

Text Format

Headings and text should look like this heading and paragraph. Use single spacing with 1" (2.54 cm) margins on ALL sides. Please indent 0.5 inch (1.3 cm) at the beginning of each paragraph and leave a space between paragraphs. Justify the text (see toolbar options and note how pretty the right margin of this paragraph lines up!). Section headings should be in bold (as above) at the left margin.



Please use the following format for figure legends

> Figure 1. Legends should appear under the photo or graph in this format in 10-point font, aligned with the sides of the image or figure (center or justify). Photographs should be pasted into the document in the proper location by the author. All photos MUST be formatted as low-resolution files, ideally no 'larger' than approximately 300 – 500 KB. I may reduce the size (appearance on the page) of figures and photographs to save space. Photos, tables, and figures not referred to in the text may be omitted for the same reason.

Table Legends

Table legends go above the table. Otherwise, formatting is as above for figures.

Other Things I Whine About

- Please don't use Paragraph formatting to add space above or below lines. I have to remove all of these. Start with a single-spaced Word template.
- Use the "enter" key for all spaces ("carriage return" for those who remember typewriters with a slidey thing on top).
- If you submit a table, put the data IN an actual table. Don't use the space bar or tabs to "line up stuff." This formatting can be lost if I have to change margins.
- Use the "tab" key to set your 0.5" indent at the start of each paragraph. It's likely your default. Don't use the space bar.
- Use bullets or numbers to make lists. It is easier to reformat these later if needed.

Short Contributions ("Ichthyological Notes")

These include any articles, observations, or points of interest that are about a page or less in length. A brief bold faced and capitalized title should be centered, the body text should be formatted as above, and **author and affiliation should be placed at the end of the piece** with the

left end of each bolded line right of the center of the page. Reformatting that must be done by the editor may reduce a shorter "main" article to a note, or may bump a note up to main article status.

Reviews, abstracts, translations (with proper permissions) and bibliographies are welcome. Humor, editoral pieces, apocrypha, and serious technical articles are equally appreciated.

RAW 2020

Details will be available soon. In the meantime, contact:

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